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The Effect of Drought Stress on Morpho-Physiological Traits of Alfalfa (*Medicago sativa* L.) Genotypes under Organic Farming

A Dissertation

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Summary

Legume fodder crops such as alfalfa (*Medicago sativa* L.) are an essential component of organic systems especially in arid and semiarid conditions. Alfalfa is usually grown as a rain-fed crop in crop rotations in organic farming systems in eastern Austria, where year-to-year fluctuations of rainfall cause different levels of drought stress. In this study, 18 alfalfa genotypes from different geographical origins, 8 Iranian ecotypes and 10 European cultivars, were evaluated under irrigated and rain-fed conditions at the research station of the University of Natural Resources and Applied Life Sciences Vienna (BOKU), Austria, during 2006-08. The objectives of study were: i) assessing of adaptability of different genotypes under irrigated and rain-fed organic farming; ii) comparing Iranian ecotypes vs. European cultivars ; iii) assessing of drought stress effects under rain-fed experiment on performance of genotypes and suitability of different drought tolerance indices for selection of superior genotypes; iv) studying of correlation between different characters in both conditions and determination of direct and indirect effects of characters on BNF, shoot and root dry matter; v) measuring genetic distance and divergence between genotypes and classifying them based on morphological and physiological characters; vi) evaluating carbon isotope discrimination (Δ) of genotypes in different plant parts and association between water use efficiency (WUE) and Δ in both conditions.

The results showed that the year, location, genotype and their interactions had significant effects on the most of studied characters. Except for leaf to stem ratio, shoot and root protein content, average of all traits was reduced under stress condition in rain-fed trial. The cultivar Sitel was the best genotype (as wide adapted genotype) across two conditions. As specific adaptation, Vlasta and Sitel can be selected for stress and non-stress condition, respectively. Among Iranian ecotypes, Mohajeran under irrigated and Ghara-aghaj and Gharghologh under rain-fed condition had the best performance.

The high yielding genotypes had taller plants and denser stands, especially under rain-fed condition. The association between irrigated and rain-fed performances increased with decreasing stress intensity (SI) from the first to the second study year. In path analysis, all direct effects of BNF components were positive in both conditions, while some of the indirect effects were negative. Plant height and LAI can be considered as primary selection criteria for improving shoot DM, while crop regrowth and plant height, with antonymous effects, were more important for improving root dry matter. Cluster analysis clearly differentiated Iranian ecotypes and European cultivars from each other using morphological and physiological data, whereas under rain-fed condition, influence of drought stress and different response of genotypes resulted in small changes in grouping of genotypes. Stress tolerance index (STI) and geometric mean productivity (GMP) were the best indices to distinguish genotypes with high performance in both conditions, while stress susceptibility index (SSI) was the best one to separate genotypes with high yield potential under rain-fed condition.

The shoot was the best and additionally simplest and most economical single criterion rather than stubble and root, to characterize alfalfa genotypes for high water use efficiency (WUE) via carbon isotope discrimination (Δ) in this study. The genotypes differed in Δ-shoot depending on harvest time and conditions (irrigated or rain-fed), indicating inconsistent differences in Δ-shoot. The rank correlations between different harvests for Δ-shoot were low and mostly insignificant under both conditions. The rank correlation between two locations or years was positive and mostly non significant for Δ in all plant parts in spite of non significant L × G, Y × G and L ×Y × G interaction. The relation between SHDM (shoot dry matter), TBY (total biomass yield) and consequently water use efficiency (WUE-SHDM and WUE-TBY) and Δ responses of genotypes (shoot, stubble and root) were variable based on plant part and study conditions (irrigated and rain-fed). Correlations between Δ-shoot and shoot dry matter, total biomass yield and their relevant water use efficiency were positive under irrigated condition, while they were negative under rain-fed condition. Regarding the variable and weak correlations, simultaneous assessment of genotypes for Δ-shoot and biomass production can ensure the selection of superior genotypes and minimize potential biomass reductions that may result from using Δ-shoot as the only selection criterion to improve WUE.

Key words: Drought stress, Biological nitrogen fixation, Shoot dry matter, Biomass yield, Repeated measure analysis, α-Lattice design, Stability analysis, AMMI analysis, Path analysis, Cluster analysis, Genetic distance, Lucerne, Iranian ecotypes, Correlation, Water use efficiency, Carbon isotope discrimination.

Zusammenfassung

Futterleguminosen wie Luzerne (*Medicago sativa* L.) sind ein essentieller Bestandteil von Betriebssystemen des Ökologischen Landbaus, vor allem unter ariden und semiariden Bedingungen. Luzerne wird in Ökologischen Landbausystemen Ostösterreichs üblicherweise unter natürlichen Niederschlagsverhältnissen, ohne Bewässerung, angebaut. Jährliche Schwankungen der Niederschläge verursachen hier Trockenstress auf unterschiedlichem Niveau. In der vorliegenden Untersuchung wurden 18 Luzerne-Genotypen unterschiedlicher geografischer Herkunft, acht iranische Ökotypen und zehn europäische Sorten, auf der Versuchswirtschaft Groß Enzersdorf der Universität für Bodenkultur Wien (BOKU), Österreich, in den Jahren 2006 bis 2008 unter bewässerten und unbewässerten Bedingungen verglichen.

Die Ziele der Untersuchung waren a) die Anpassungsfähigkeit unterschiedlicher Genotypen unter bewässerten und unbewässerten Bedingungen im Ökologischen Landbau festzustellen, b) iranische Ökotypen und europäische Sorten miteinander zu vergleichen, c) die Auswirkungen von Trockenstress in dem Versuch ohne Bewässerung auf die Leistungsfähigkeit der Genotypen und die Eignung verschiedener Indices für Trockentoleranz als Kriterien der Selektion überlegener Genotypen festzustellen, d) die Korrelation zwischen unterschiedlichen Merkmalen unter den beiden Bedingungen zu untersuchen und direkte sowie indirekte Wirkungen von Merkmalen auf die biologische Stickstoffbindung (BNF) sowie die Spross- und Wurzeltrockenmasse zu bestimmen, e) die genetische Distanz und Abweichung zwischen den Genotypen zu messen und diese auf der Basis morphologischer und physiologischer Merkmale zu klassifizieren und f) die Kohlenstoffisotopen-Diskriminierung (Δ) der Genotypen in verschiedenen Pflanzenteilen festzustellen und ihren Zusammenhang mit der Wassernutzungseffizienz unter den beiden genannten Bedingungen zu bestimmen.

Das Jahr, der Ort, die Genotypen und die Wechselwirkungen dieser Faktoren hatten auf die meisten untersuchten Merkmale signifikante Auswirkungen. Mit Ausnahme des Blatt-Stängel-Verhältnisses sowie der Spross- und Wurzel-Proteingehalte waren die Mittelwerte aller Eigenschaften unter Stressbedingungen im Versuch ohne Bewässerung verringert. Die Sorte Sitel war der über beide Bedingungen hinweg am besten angepasste Genotyp. Aufgrund ihrer spezifischen Anpassung, können die Sorten Vlasta unter Stressbedingungen (ohne Bewässerung) und die Sorte Sitel unter Bedingungen ohne Trockenstress (mit Bewässerung) gewählt werden. Unter den iranischen Ökotypen erzielten Mohajeran unter bewässerten

Bedingungen sowie Ghara-aghaj und Gharghologh unter natürlichen Niederschlagsverhältnissen die besten Ergebnisse.

Die Genotypen mit den höheren Erträgen hatten eine größere Pflanzenhöhe und dichtere Bestände, vor allem unter natürlichen Niederschlagsverhältnissen. Der Zusammenhang zwischen den Ergebnissen, die unter bewässerten Bedingungen und unter natürlichen Niederschlagsverhältnissen erzielt wurden, war im zweiten Untersuchungsjahr bei geringerer Stressintensität (SI) enger als im ersten Jahr. In der Pfadanalyse waren alle direkten Effekte der BNF-Komponenten unter beiden Bedingungen positiv, wogegen einige der indirekten Effekte negativ waren. Die Pflanzenhöhe und der Blattflächenindex (LAI) können als die hauptsächlichen Selektionskriterien zur Verbesserung der Sprosstrockenmasse angesehen werden. Der Pflanzen-Wiederaufwuchs und die Pflanzenhöhe, mit gegenteiligen Wirkungen, waren dagegen bedeutender für die Verbesserung der Wurzeltrockenmasse. Eine Clusteranalyse, basierend auf morphologischen und physiologischen Daten, unterschied die iranischen Ökotypen klar von den europäischen Sorten. Unter natürlichen Niederschlagsverhältnissen bewirkten der Trockenstress und unterschiedliche Reaktionen der Genotypen kleinere Veränderungen in der Gruppierung der Genotypen. Der Stresstoleranz-Index (STI) und das Geometrische Mittel der Produktivität (GMP) waren die besten Indices, um Genotypen mit hoher Leistung unter beiden Bedingungen zu erkennen. Dagegen war der Stressempfindlichkeits-Index (SSI) das beste Mass, um Genotypen mit hohem Ertragspotential unter natürlichen Niederschlagsverhältnissen zu identifizieren.

In dieser Untersuchung war der Spross, im Vergleich zu Stoppel und Wurzel, das beste, einfachste und ökonomischste Einzelmerkmal, um Luzerne Genotypen mittels Kohlenstoffisotopen-Diskriminierung (Δ) in ihrer Wassernutzungseffizienz zu charakterisieren. Die Genotypen unterschieden sich im Δ-Spross in Abhängigkeit von Erntezeitpunkt und Bedingungen (bewässert oder nicht). Diese Unterschiede in den Δ-Spross-Werten waren inkonsistent. Die Rangkorrelationen zwischen verschiedenen Ernten für die Δ-Spross-Werte waren unter beiden Bedingungen gering und zumeist nicht signifikant. Die Rangkorrelation zwischen den beiden Orten oder Jahren für die Δ-Werte in allen Pflanzenteilen waren positiv und zumeist nicht signifikant, obwohl die $L \times G$-, $Y \times G$- und $L \times Y \times G$-Wechselwirkungen nicht signifikant waren. Die Beziehung zwischen Sprosstrockenmasse (SHDM), Gesamtbiomasse (TBY) sowie den entsprechenden Wassernutzungseffizienzen für Sprosstrockenmasse (WUE-SHDM) und Gesamtbiomasse (WUE-TBY) einerseits und den Δ-Werten von Spross, Stoppel und Wurzel der Genotypen andererseits variierten mit den Pflanzenteilen und Untersuchungsbedingungen (mit bzw. ohne

Bewässerung). Die Korrelationen zwischen Δ-Spross-Werten, Sprosstrockenmasse, Gesamtbiomasse und ihrer jeweiligen Wassernutzungseffizienz waren unter bewässerten Bedingungen positiv, unter natürlichen Niederschlagsverhältnissen aber negativ. Vor dem Hintergrund variabler und schwacher Korrelationen kann das gleichzeitige Erfassen von Δ-Spross-Werten und Biomasseproduktion der Genotypen die Selektion überlegener Genotypen sichern und mögliche Biomasseverluste minimieren, die sich bei Verwenden der Δ-Spross-Werte als einziges Selektionskriterium zur Verbesserung der Wassernutzungseffizienz ergeben können.

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Abbreviations

$\Delta^{13}C$	Carbon isotope discrimination
AMMI	Additive main effects and multiplicative interaction
ANOVA	Analysis of variance
ASV	AMMI stability value
BNF	Biological nitrogen fixation
CHL	Chlorophyll content
CP	Crude protein
CV_i	Coefficient of variability
FC	Field capacity
FDR	Frequency Domain Reflectometry
GEI	Genetic by environment interaction
GMP	Geometric mean productivity
IFOAM	International Federation of Organic Agriculture Movements
LAI	Leaf area index
LSR	Leaf to stem ratio
MP	Mean productivity
NN	Node number per stem
PCA	Principal component analysis
PH	Plant height
P_i	Superiority statistic
PWP	Permanent wilting point
RODM	Root dry matter
SAW	Soil available water
SHDM	Shoot dry matter
SI	Stress intensity
SSI	Stress susceptibility index
STDM	Stubble dry matter
STI	Stress tolerance index
STN	Stem number
TOL	Tolerance
WUE	Water use efficiency
$б_i^2$	Stability variance

Preface

Organic agriculture as a significant and effective alternative to conventional, chemical-based agriculture is often characterized as a natural way of farming, mostly referring to the absence of synthetic chemical inputs, such as chemical fertilizers, herbicides, and pesticides (IFOAM, 2002). According to the survey on organic farming worldwide (2006), almost 30.4 million hectares are managed organically by more than 700’000 farms (Willer *et al*., 2008). Nitrogen (N) is one of the major limiting nutrients for most crops, contributing to reduced agricultural yields throughout the world. Organic farming aims to be self-sufficient in nitrogen (N) through fixation of atmospheric N_2 by legumes, recycling of crop residues and application of manures or composts. Legume fodder crops are an essential component of organic system especially in arid and semiarid conditions. The appropriate selection of legume species and cultivars in rotations plays an important role and helps organic farming in its aims especially for nitrogen self-sufficiency. Alfalfa or Lucerne (*Medicago sativa* L.) is the world’s most important forage crop (Barnes *et al*. 1988) that provides high-quality forage, fixes atmospheric nitrogen, exhibits rapid growth after defoliation, and survives in dry, high-temperature environments (Johnson and Rumbaugh 1995).

Drought, as a major production constraint, reduces crop performances and productivity in drought-prone environment. Low and inconsistent precipitation in rain-fed farming; insufficient and irregular irrigation under irrigated farming lead to different levels of drought stress during crop growing seasons. Development and utilization of drought tolerant cultivars will decrease yield reductions and enhance biomass production. In addition, the use of morphological and physiological characters in indirect selection and improvement for key economical and biological characters such as forage yield, water use efficiency and biological nitrogen fixation enhance precision and efficiency of selection and decrease time and costs of execution of breeding programs. This study was planned (i) to survey different morphological and physiological characters such as biomass production, water use efficiency, BNF and their interrelationships in different alfalfa genotypes under irrigated and rain-fed organic farming; (ii) to select the best genotypes based on different drought tolerance indices and crop performance stability parameters; and (iii) to evaluate association between carbon isotope discrimination and water use efficiency and using this trait in indirect selection for high WUE. The results of this study will be presented in three separate chapters.

Chapter one

The Response of Iranian and European Alfalfa (*Medicago sativa* L.) Genotypes to Different Conditions in Organic Farming

The Response of Iranian and European Alfalfa (*Medicago sativa* L.) Genotypes to Different Conditions in Organic Farming

Abstract

Legume fodder crops such as alfalfa (*Medicago sativa* L.) are an essential component of organic systems especially in arid and semiarid conditions. Alfalfa is the best known fodder crop with high ability of biological nitrogen fixation (BNF) and drought tolerance in dry, pannonian region of east Austria. In this study, different morphological and physiological characters of 18 alfalfa genotypes from different geographical origins, 8 Iranian ecotypes and 10 European cultivars, were evaluated under irrigated and rain-fed conditions at research station of the University of Natural Resources and Applied Life Sciences (BOKU), Vienna, Austria, during 2006-08. The objectives of study were : i) assessing of adaptability of different genotypes for organic farming and determining of the best genotype/genotypes in two mentioned conditions based on biomass production, BNF and their stability; ii) comparing Iranian ecotypes vs. European cultivars based on different characters in organic farming; iii) assessing of drought stress effects under rain-fed experiment on performance of genotypes; iv) studying of interrelationships among different characters in different conditions and determination of direct and indirect effects of characters on BNF, shoot and root dry matter; v) measuring genetic distance and divergence between genotypes and classifying them based on morphological and physiological characters. The analysis of variance showed significant differences for main factors of year, location, genotype and their interactions in the most of studied characters. Except for leaf to stem ratio, shoot and root protein content, average of all traits was reduced under stress condition in rain-fed trial. With regard to mean comparisons and stability analysis for shoot dry matter, total biomass yield and BNF, the cultivar Sitel was the best genotype followed by PlatoZS, Fix232, Vlasta and Gharghologh. Although, AMMI analysis was found to be more informative in describing the adaptive response of the genotypes, P_i (superiority measure) was the best stability parameter to select high yield and stable genotype/ genotypes based on simplicity of calculation and correlation with crop performance in this study. Regarding to correlation coefficients, it is concluded that the high yielding genotypes had taller plants and denser stands, especially under rain-fed condition. In path analysis, all direct effects of BNF components were positive in both conditions, while some of indirect effects were negative. Plant height and LAI can be considered as primary selection criteria for improving shoot DM, while crop regrowth and plant height, with antonymous effects, were more important for improving root dry matter. Cluster analysis clearly differentiated Iranian ecotypes and European cultivars from each other using morphological and physiological data, whereas under rain-fed condition, influence of drought stress and different response of genotypes resulted in small change in grouping of genotypes.

Key words: Drought stress, Biological nitrogen fixation, Biomass yield, Repeated measure analysis, α- lattice, Stability analysis, AMMI analysis, Path analysis, Cluster analysis, Genetic distance.

Introduction

Alfalfa or Lucerne (*Medicago sativa* L.) is the world's most important forage crop (Barnes *et al*. 1988) and is the only forage known to have been cultivated before recorded history (Michaud *et al*. 1988). The genus *Medicago* comprises more than 60 species, which grow over a wide area stretching from China to Spain and from Sweden to North Africa (Lesins and Lesins 1979). The primary centre for the genus is in the Caucasus, north-western Iran and north-eastern Turkey (Ivanov 1977). Cultivated lucerne is autotetraploid (2n = 4x = 32), outcrossing and widely adapted perennial legume that provides a high-quality forage, fixes atmospheric nitrogen, exhibits rapid growth after defoliation, and survives in dry, high-temperature environments (Johnson and Rumbaugh 1995). Alfalfa is a versatile crop and can be used for pasture, hay, silage, green-chop, soil improvement, and soil conservation.

World lucerne areas in the 1980s were estimated at 32 million ha, of which 70% were located in the USA, USSR, and Argentina collectively (Michaud *et al.* 1988). Its importance in Europe is expected to continue, due to its contribution to sustainable agriculture and its productivity of feed proteins per unit area that is the greatest among the forage or grain legumes (Huyghe, 2003).

Organic agriculture is developing rapidly, and statistical information is now available from 138 countries of the world. According to the survey on organic farming worldwide (2006), almost 30.4 million hectares are managed organically by more than 700'000 farms (Willer *et al*., 2008). Organically cultivated area in Europe was about 7.4 million hectares which is 24 percent of the world's organic land (Willer *et al*., 2008). Organic agriculture is often characterized as a natural way of farming, mostly referring to the absence of synthetic chemical inputs, such as chemical fertilizers, herbicides, and pesticides (IFOAM, 2002). In 2007, the FAO recognized organic agriculture as a significant and singularly effective alternative to conventional, chemical-based agriculture (Willer *et al*., 2008 pp 231).

Nitrogen (N) is one of the major limiting nutrients for most crops and other plant species (Newbould, 1989), contributing to reduced agricultural yields throughout the world. Organic farming aims to be self-sufficient in nitrogen (N) through fixation of atmospheric N_2 by legumes, recycling of crop residues and application of manures or composts. Biological N_2 fixation (BNF) is an important aspect of sustainable and environmentally friendly food production and long-term crop productivity (Montanez 2000). Essentially all agriculturally important legume species have the ability to symbiosis with a group of bacteria collectively known as rhizobia. In this symbiosis, the bacteria derive energy from the host for growth and

N_2 fixation, and are protected from external stresses; the host accesses a form of nitrogen it could not otherwise utilize (Graham *et al*. 2004). Legume fodder crops such as alfalfa are an essential component of organic system especially in arid and semiarid conditions. Worldwide some 44 to 66 million tons of atmospheric N_2 are fixed annually, providing nearly half of all the nitrogen used in agriculture (Graham *et al.* 2004).

Stockless organic farming is predominant in the dry, pannonian region of the eastern Austria. Alfalfa is the best known fodder crop with high ability of BNF and drought tolerance in eastern Austria (Pietsch 2004). Alfalfa can play an important role in the crop-livestock organic systems of southern Europe, because of its adaptation to low input or drought stressed conditions, its positive effect on the nitrogen balance and the soil fertility, and the high protein content and quality of its forage (Campiglia *et al*., 1999; Huyghe, 2003; Annicchiarico *et al*., 2006). From an agronomic perspective, alfalfa is a great rotational crop because of its soil conditioning abilities.

A complete organic production chain needs to use varieties that have been bred typically for organic conditions. Currently, organic farmers largely depend on varieties supplied by conventional plant breeder, even though organic farming conditions demand varieties with different characteristics than conventional varieties (Lammart van Bueren *et al.* 1999). The occurrence of cultivar by environment interaction between conventional and organic management may also justify the selection of material specifically targeted to organic systems (Annicchiarico *et al*., 2006). As an alternative, existing varieties can be assessed in organic conditions and the best ones recommended based on values for organic conditions and farmers until achieving new organically bred varieties. Forage yield trials are widely used tool for breeders to select suitable genotypes for registration, recommendation (Boller *et al*. 2006) and needed material for future breeding program. However, an example of a variety specifically bred in Italy for use in organic agriculture is the registered Lucerne ‘Cuore Verde’ (Falcinelli and Torricelli, 2004). Cultivars in organic agriculture should have the ability to perform under low input of organic fertilizers, a good root system, the ability to interact with beneficial soil microorganisms and to suppress weeds, and the ability to produce a healthy crop and healthy propagules (Lammerts van Bueren *et al*., 2002, 2003). The correct choice of variety is one of the most important aspects to consider; especially in organic farming where the genetic characteristics could solve problems due to biotic stress (Torricelli 2006). There is generally slight interest for fodder legume crops, like alfalfa, to breed specifically for organic systems. Casler *et al.* (2006) reported that there are no exclusive efforts for development of fodder crop cultivars for organic agriculture in USA, due to long term reductions in the infrastructure and personnel included in fodder crop breeding. However, Annicchiarico *et al*. (2006) found that with respect to weed competition at the first year and first harvest of second year of alfalfa, the specific breeding of alfalfa for organic systems is not required, because of the consistency of well-performing material between organic and chemically-weeded managements. They reported that cultivar by environment interaction effects and their implications on selection and recommendation of alfalfa varieties for Italian environment are more affected by the geographical area and the drought stress level than by cropping in conventional or organic farming.

Principally breeding for organic farming is not completely different from breeding for conventional farming. Additionally traits with higher priority in organic farming may have lower priority in conventional farming and vice versa (Lammerts van Bueren *et al.* 2002). Therefore, identification of effective characters, their interrelationships and relative importance in improvement of target characters such as dry matter yield is the key step and essential part of any breeding program, especially for organic farming. The identification of single adaptive traits or combinations of traits (plant architectures) associated with better yield response may increase the efficiency of selection for wide or specific adaptation, integrating adaptive trait-based and yield-based selection to detect more precisely or in earlier selection stages the best-performing material (van Oosterom *et al*., 1996; Annicchiarico, 2002a). The traits can directly - due to trait per se - or indirectly - due to correlation between traits - influence target characters. These effects can be delineated by means of path analysis. By this method, correlations between target character and others are partitioned into direct and indirect effects (Dewey and Lu, 1959; Kang *et al*., 1983; Scheiner *et al.*, 2000). Like in other crops, this method has frequently been used in alfalfa especially for forage dry matter and quality (Kephart *et al*., 1992; Koslov *et al*., 1994; Popovic *et al*., 2006; Berg *et al.*, 2007).

Genetic structure of alfalfa is complex at both individual and population levels because of being autotetraploid, allogamous and a seed-propagated species. Information about germplasm diversity and relationships among elite breeding materials is of fundamental importance in plant breeding (Hallauer and Miranda 1988). This is especially true for a species like alfalfa which suffers severe inbreeding depression. Katepa-Mupondwa *et al.* (2002) stated that researcher have postulated that multi-allelic loci are important in conditioning maximum productivity in autotetraploid alfalfa, and conversely that the loss of multi-allelic loci contributes significantly to inbreeding depression (Carnahan, 1960; Demarly, 1960; Dessureaux 1976a; Dundier and Bingham 1975). Dessureaux (1976b, 1977a) suggested the use of four unrelated broad-based populations to increase the probability of four unrelated superior alleles occurring at one locus (Cited in Katepa-Mupondwa *et al*., 2002). Therefore, genetic diversity of initial selection materials is essential for successful breeding and creation of new cultivars. For the estimates of genetic diversity, different criteria, such as morphological, agronomic and physiological characters, pedigree records, molecular markers or a combination of criteria are used. Alfalfa is distributed worldwide and grown in highly contrasting environments. This extensive geographical adaptation promotes genetic variation and gives us the opportunity to use diverse gene pools (Tucak *et al*. 2008). Cluster analysis can be applied to measure genetic distance and divergence between genotypes which can be useful in planning of the crossing program of a breeding project (Bauchan *et al.*, 1993; Riday *et al.* 2003; Dehghan-shoar *et al.*, 2005; Tucak *et al*., 2008).

Stability in ecosystems is a measure of resilience, or ability of the system to recover from a disturbance, and the resistance of the system to change (Schowalter, 2006). If an agro-ecosystem recovers quickly after a disturbance or resists stress, it is considered more stable than one that does not. Productivity is a measure of ecosystem function. In agro-ecosystems, productivity is usually measured as the yield of a crop (Barbercheck 2009). As the environmental component in organic agriculture is more variable than in conventional agriculture, yield stability is more important than yield as such (Lammerts van Bueren 2006). Genotype by environment interaction (GEI) usually occurs when different genotypes are assessed across diverse environments. GEI refers to changing of genotype ranks or values across a range of environments. The GEI complicates the selection of superior genotypes (Magari and Kang, 1993; Ebdon and Gauch, 2002). Other undesirable effects of GEI may include masking of the potential utility of exotic material (Giauffret *et al.*, 2000). However, GE interaction also provides opportunities for yield improvement. Exploring positive GE interaction while avoiding negative aspects could provide substantial opportunity for further improvement in food production worldwide (Yan and Hunt 1998). Plant breeders and agronomists often ignore GE interactions and usually select genotypes on the basis of their mean performance across environments, especially when all the test environments fall within some defined target environment. Under poor environment conditions or low input farming like organic farming, the recommendation of high yielding but unstable genotype can result in crop failures. Most agronomically and economically important traits, such as forage yield or biological nitrogen fixation (BNF), are quantitative in nature and present GEI. By growing cultivars in different environments, the highest yielding and most stable cultivars can be identified (Lu'quez *et al*., 2002). Thus, the stability of performance is one of the important properties to select consistent-performing and high-yielding genotypes in any conventional or organic farming systems. Improved adaptation and yield stability may derive in the long term from the definition of an appropriate breeding strategy, and in the short term from the appropriate choice of cultivars (Annicchiarico 2002). Lotti *et al.* (2008) evaluated genetic resources and stocks of durum wheat, tomato and pea for high stability in low input systems by means of organic practices. As shoot dry matter, GEI can occur for other important traits such as BNF or root dry matter. Zobel (1992b) expressed that root traits typically have a considerably larger GE interaction than shoot traits. However, Annicchiarico (2007) found no GE interaction for root DM in alfalfa, indicating that the attitude towards greater or smaller root development is a genetic characteristic of the cultivar which is expressed consistently in different environments independently of its adaptive value.

Many statistics have been suggested to determine stability of genotypes (Lin *et al*., 1986; Becker and Leon 1988, Flores *et al*., 1998; Hussein *et al*.2000). Flores *et al.* (1998) divided 22 different methods of analyzing GE interaction and stability parameters into 3 categories: univariate parametric, univariate nonparametric and multivariate methods. Lin *et al.* (1986) classified nine univariate parametric stability statistics in 4 groups and 3 types. Based on 3 types of stability statistics, they represent 3 concepts of stability in which a genotype can be considered as stable if 1) its environment variance is small, 2) its average yield is parallel to overall mean of all genotypes included in trial and 3) its residual mean square from a regression on the environmental index is small. Francis and Kannenberg (1978) used the conventional coefficient of variation CV% of each genotype as a stability measure which is placed in type 1 stability category and concept. Shukla (1972a) proposed the parameter of stability variance ($б_i^2$) as stability measure which is an unbiased estimate of the variance of a genotype across environments. Shukla's parameter is grouped in type 2 stability concept. In addition to Lin *et al.* (1986), Lin and Binns (1988) developed a superiority measure of genotypic performance, P_i, defined as the mean square distance between the genotype's response and the maximum response averaged over all environments. Hühn (1996), Nassar and Hühn (1987), Kang (1988), Ketata *et al*., (1989) and Fox *et al.* (1990) have suggested different univariate nonparametric stability statistics. Nonparametric stability measures are unaffected by data distribution. These methods are based on ranks of genotypes and a genotype is considered stable if its ranking is relatively constant across environments.

Additive main effects and multiplicative interaction (AMMI) analysis is widely used as one of the multivariate methods of GE analysis (Zobel 1988, Crossa *et al.*, 1990, Annicchiarico 1997, 2007a and b, Edbon and Gauch 2002, Flores *et al*., 1998, Tarakanovas and Sprainaitis 2005, Li *et al.* 2006). Zobel (1992b, 1994) found AMMI helpful in comprehending large interactions for root traits and in relating root systems to stress resistance. Various papers have reviewed details of AMMI analysis and its advantages and weakness (Gauch 1992, Gauch and Zobel 1996, Kang and Gauch 1996; van Eeuwijk 1995, Yan and Hunt 1998, Gauch 2006a, Yan *et al*. 2007 and Gauch *et al*. 2008). The AMMI model combines ordinary analysis of variance (ANOVA) to partition the genotype main effects, environment main effects, and genotype-by-environment (GE) interaction effects, followed by principal component analysis (PCA) to partition the GE interaction into several components (Zobel et al., 1988, Gauch 1992 and Gauch and Zobel 1996). The AMMI quantified environmental and genotypic scores (IPCA scores) that are usually shown in the form of biplots (Gabriel 1971) which are called AMMI biplots (Gauch 1992, Yan and Hunt 1998). Using the biplot technique, the genotypes and the environments can be mutually classified at the same time (Yan and Hunt 1998). The IPCA scores of a genotype in the AMMI analysis are an indicator of the stability of a genotype over environments. Selection of stable genotypes based on different component scores, i.e. IPCA1 or IPCA2 scores get different results because of their different values. Purchase (1997) suggested AMMI stability value (ASV) which is calculated using a principle of the Pythagoras theorem. Farshadfar (2008) used ASV as stability parameter to select stable wheat cultivars.

Dry matter yield is the most considered objective in forage crops breeding. Moreover, shoot dry matter has been the main indicator in determination of adaptability of alfalfa genotypes to given environment or condition (Torricelli 2006; Annicchiarico *et al*., 2006). Veronesi *et al*., (2006) expressed that dry matter yield is still the most important breeding target for lucerne and therefore deserves particular attention. Alfalfa yield components are number of plants per area, number of stems per area, weight and height of individual stem (Fick *et al*., 1988). The rate of genetic improvement for dry matter yield in alfalfa has been lower than the rate in grain crops (Hill *et al.*, 1988; Brummer 1999; Skinner *et al.*, 2000). In addition to autotetraploid nature of alfalfa, because of quantitative inheritance of shoot dry matter yield, high influence of environment in its expression and low heritability, improvement and selection for high DM yield has been indirectly done by yield components and other properties such as biotic and abiotic stress tolerance.

BNF can be regarded as one of the most important properties of alfalfa cultivars for organic systems. Main and target traits in legume fodder crop improvement such as dry matter yield, abiotic and biotic stress tolerance, protein content can be considered as different characters associated to quantity N_2 fixation. Genetic variation exists within many legume species for the amount of atmospheric N that can be fixed by the legume-rhizobium interaction (Casler *et al.*, 2007). Cultivar variation in different traits associated with N_2 fixation has been demonstrated in many legume such as clover (Nutman 1967), soybean (Hardy *et al.* 1973; Pazdernik *et al.* 1996), common bean (Graham and Rosas 1977; Rennie and Kemp 1983), and alfalfa (Degenhart *et al.* 1992; Jessn *et al.*, 1988). However, there is no simple and easy approach to increase BNF in legumes grown as part of a cropping system, under field conditions. Numerous factors play an important role in controlling BNF and the amount of nitrogen fixation by legumes such as climatic variables, soil-physical properties, agronomic management, host-rhizobia combination and socioeconomic aspects (Montanez 2000).

There are different techniques to estimate nitrogen fixation (Boddey 1987; Peoples *et al.*, 1989a; Hansen 1994; Chalk and Ladha 1999; Giller 2001). There is no single, incontestably correct method to measure nitrogen fixation, because all techniques available have various benefits and obstacles (Hanson 1994). The techniques can be generally divided into two groups, direct and indirect (Giller 2001; Hanson 1994). Direct methods utilize nitrogen itself to determine N_2 fixation, while indirect techniques are based on alternative substance which can be used as indicators of N_2 fixation or the activity of the enzyme nitrogenase. Nitrogen-difference and isotope-based (^{15}N enrichment and natural abundance) methods are considered as direct method. Giller (2001) concluded that isotope-based methods provide the best approach for integrated measurements of N_2 fixation in plant provided that the assumptions of methods are fulfilled. For crop improvement programs, however, the N-difference method which measures N accumulation in the growing legume and reference crops is the most practical especially in the soils with poor capacity to supply mineral N such as organic systems. Loges *et al.* (2000) concluded that estimation of nitrogen fixation by different methods will lead to different data and choice of estimation method slightly affects the ranking between the experimental treatments. However, there are strong correlation between legume yield and nitrogen fixation regardless of estimation method. Brunner and Zapata (1984) for example found that symbiotic nitrogen fixation was closely correlated with total plant top biomass and nitrogen yield in field bean (*Vicia faba* var. Minor).

Forage quality is another important trait in fodder crops in general and in alfalfa breeding in particular. The term forage quality is a broad term referring to a number of factors that affect nutritive value of the forage. Posselt (1994) considered dry matter digestibility as main factor affecting nutritive value and quality of forage. There is a positive relation between digestibility and protein content in alfalfa (Ray *et al.* 1999). Therefore, protein content can be taken into account as a main component and indicator of forage quality (Rotili *et al.,* 2001) which is mainly related to the leaf to stem ratio, leaf age and health at harvesting time (Scotti *et al.*, 2006). Rotili *et al.* (1992) expressed that protein content variation among varieties is mainly related to the stratigraphy of the alfalfa stand and its developmental stage. There is usually negative correlation between forage yield and quality (Hill *et al.* 1988).

Drought is a major production constraint, reducing crop yields in a drought-prone environment. Drought affected crop productivity nearly as much as all the other environmental factors combined. Irrigation has been one solution but is becoming less so as global water demand increases (Boyer and Westgate 2004). Development of drought tolerant crops will decrease yield reductions and enhance food production. Crop drought resistance is a major factor in the stabilization of crop performance in drought-prone environments (Blum 1999). Extended drought accompanied by rather high temperatures may severely curtail yields especially in shallow soils or soils with low water holding capacity (Donovan and Meek 1984). From a plant breeding point of view, drought resistance can be defined based on the relative yield or survival of a genotype in comparison to other genotypes in the same condition. The genotypic rankings are very dependent on the range of environments chosen in screening of genetic materials for overall yield under dry conditions (Hall *et al.*, 2005; Atlin *et al.*, 2006). Breeding for drought tolerance has produced improved cultivars for drought-prone environments, but progress has been slow due to the complex physiological responses to drought, various environmental factors, and their interactions (Nguyen 1999). Plant breeders have made some progress by combining specific drought-tolerance characters such as improved water use efficiency (Condon *et al.*, 2004) into commercial lines that perform well under specific drought conditions (Jones 2007). Water availability is usually the main factor controlling yield of lucerne (Frame *et al.*, 1998). Although drought stress reduces its yield, lucerne has the greatest yield potential in dry conditions among perennial legumes (Peterson *et al.*, 1992). Soil water deficits have been shown to reduce alfalfa yields (Lucey and Tesar 1965, Carter and sheaffer 1983; Wilson *et al*., 1983). Additionally, Halim *et al*. (1989) concluded that the slowing of plant maturation and growth during water stress accounted for much, but not all, of the changes in forage quality, while leaf to stem ratio increased from 0.60 in the well-watered treatments to 0.72 in the most severely stressed treatment. It seems that reduction of stem growth and biomass is main reason in increasing of leaf to stem ratio. The effect of drought stress on BNF has been widely reviewed and reported (Zahran, 1999; Marino *et al.*, 2007). Drought affects the fixation process and the amount of N_2 fixed by influencing symbiosis between host and rhizobia, rhizobial survival in soil, the host and the process of nodulation itself (Graham, 1992). BNF is of great importance in low-input and stress-prone environments due to enhancement of plant growth and DM production without N mineral input, low-cost source of proteins, and improvement of soil fertility for the benefit of companion plants or following crops. In addition, organic farmers strive for multi-faced solutions to spread risks, by including additional morphological and physiological traits that can contribute to an acceptable level of field tolerance against stress conditions (Lammerts van Bueren 2006). Drought stress also results in a reduction of root DM (Abdul- Jabbar *et al*., 1984; Salter *et al*., 1984; Annicchiarico, 2007). Annicchiarico (2007) found that the mean plant response to drought-stress was a reduction of root biomass, but the cultivars specifically adapted to these conditions were characterized by a further, genetically based root reduction.

Increasing in demand for organic dairy feed makes alfalfa a suitable crop for some organic farmers. So, management practices or utilization system of above-ground produced biomass will also affect the N_2 fixation quantity and process. Utilization systems (e.g. forage use versus green manure) strongly affect the amount of N supplied by the legume crops. In organic farming systems nitrogen is accumulated by legume crops in rotation and design of crop rotation is strongly related to amount of N fixed by legumes. In a forage use management, most of the fixed N_2 is removed by harvesting the forage legumes, reducing the benefit to the subsequent crops (Pietsch *et al*., 2007). In such a system, the amount of non-harvestable part of total biomass yield (stubble and root) can play important role to supply N for following crops. The proportion of the non-harvestable part, in particular root, can be about half of the total biomass yield, depending on soil, climate, cropping management and genotype factors. Peoples *et al.* (2009) stated that 30-60% of the legume's total plant N may be below-ground associated with roots and nodules. Consequently crop legume residues can still contain significant amounts of fixed N even after a large amount of N is removed at harvest. Loges *et al.* (2000) reported 15 – 227 % increase in BNF estimation based on total plant material plus soil mineral N content compared to harvestable plant material only in different management practices, seed mixtures and estimation methods. In contrast, Xu *et al.* (2007) found that alfalfa root biomass only contributed to 2.3 % of total biomass yield which was responsible for the higher yearly above-ground WUE of alfalfa. In addition, roots play an important role in maintaining water and nutrient supply to plant tissues, and they also contribute in the maintenance of soil organic matter content and structure (Pietola and Alakukku, 2005). Root development is an important indicator for estimating suitability to drought resistance (Lu 1992; Ren 1998). Strong root development is a characteristic of lucerne cultivars adapted to dryland conditions (Guo *et al.* 2002). On the contrary, Annicchiarico (2007) stated an extensive root is not necessarily related to adaptation to drought-prone environments. Therefore, root yield can be considered as a selection criteria as well as shoot yield to recommend suitable cultivar in organic systems especially for forage use management in drought-prone environment. Guo *et al*. (2004) determined the suitability of lucerne cultivars, with respect to root development in semi-arid environment of west China. However, root study and breeding have received less attention than shoots because of the difficulties of extraction and quantification as well as farmer consideration in above-ground biomass production. Indirect selection for improvement of different root properties such as biomass yield can be considered as alternative way, with regard to different difficulties in root study and breeding programs. Johnson *et al.* (1998) reported variation between different alfalfa genotypes for root morphological traits and also correlation between fall dormancy and studied traits. They concluded that variation observed for root morphological traits among evaluated alfalfa entries indicated that selection for specific root modifications could be effective.

In this study, different morphological and physiological characters of 18 alfalfa genotypes from different geographical origins were evaluated under irrigated and rain-fed conditions of dry, pannonian region of east Austria with the objectives of : i) assessing of adaptability of different genotypes for organic farming and determining the best genotype/genotypes under the two mentioned conditions based on biomass production, BNF and their stability; ii) comparing Iranian ecotypes vs. European cultivars based on different characters in organic farming; iii) assessing drought stress effects under the rain-fed condition on performance of genotypes; iv) studying interrelationships among different characters under different conditions and determination of direct and indirect effects of characters on BNF, shoot and root dry matter; v) measuring genetic distance and divergence between genotypes and classifying them based on morphological and physiological characters.

Materials and Methods

Site and experiment description

In order to assess the performance of different alfalfa genotypes in organic conditions and the effects of drought stress on biomass production and BNF, this study has been carried out in two separate trials, namely, irrigated (normal) and rain-fed (drought stress) at two different organically managed fields, Gross-Enzersdorf and Raasdorf, respectively, of research station of the University of Natural Resources and Applied Life Sciences (BOKU), Vienna, Austria. The farm managements were organic, stockless and no organic manures were applied. The soils are a Calcaric Phaeozem (WRB) from loess with a silty loam textures. Some properties of soils are shown in Table 1. The soils are described in detail in Freyer *et al.*, (2000) and

Pietsch *et al.,* (2007). Before planting in the experiment at Gross-Enzersdorf (irrigated trial), the soil hydraulic properties of field capacity (FC) and permanent wilting point (PWP) were determined. The average annual precipitation (1971-2000) was 520 mm. The amount of precipitation, average temperature and applied irrigation water is shown in Figure 1 from March to September in 2007-8.

Table 1. Some properties of the experimental soil at two field trials.

	Gross-Enzerdorf (Irrigated)	Rassdorf (Rain-fed)
Texture	Silty loam	Silty loam
Organic carbon content (%)		
0-30 cm	1.5	2.0
30-60 cm	1.4	0.7
Depth of A horizon	45-50cm	25-35cm
Bulk density (g cm^{-1})	1.4-1.6	1.3-1.4

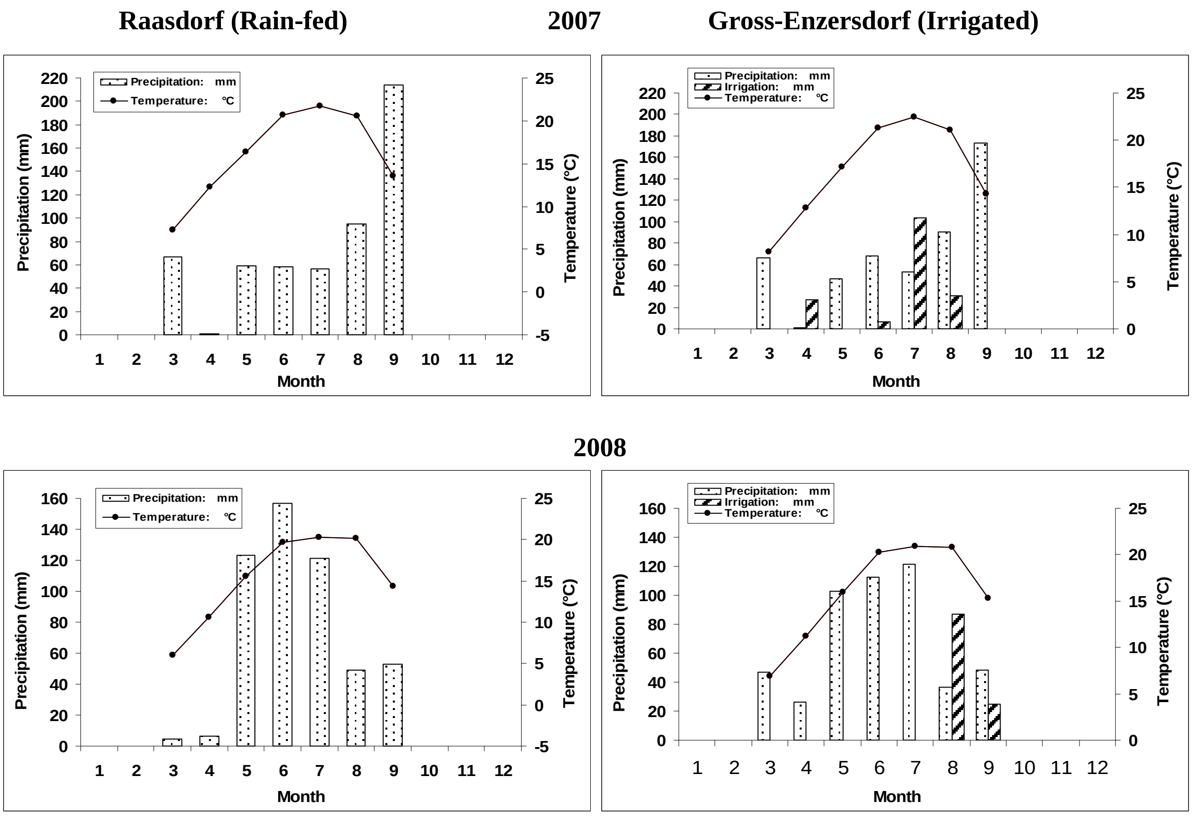


Fig. 1. Monthly precipitation, average temperature and applied irrigation water from March to September 2007 and 2008.

Experimental treatment and design

Eighteen alfalfa cultivar and ecotypes (Table-2) containing eight Iranian ecotypes and ten European varieties were evaluated in two different conditions, irrigated in Gross-Enzersdorf (48º12' N, 16º33' E) and rain-fed in Raasdorf (48º15' N, 16º37' E), during 2006-08.

Table 2. Name of tested genotypes, reference crops and their origin.

Variant	Alfalfa varieties, grass species[1]	Origen	Germination rate (%)
1	Mohajeran	Iran-West	92.0
2	Khorvande	Iran-West	92.0
3	Famenin	Iran-West	92.5
4	Gharghologh	Iran-Northwest	88.5
5	Ordobad	Iran-Northwest	94.5
6	Shorakat	Iran-Northwest	94.4
7	Ghara-aghaj	Iran-Northwest	86.5
8	Hokm-abad	Iran-Northwest	87.5
9	Sitel	Netherlands	81.0
10	Verko	Hungry	97.5
11	Vlasta	Czech Republic	95.0
12	Monz42	Slovakia	85.0
13	Fix232	Slovakia	92.0
14	NS- Banat	Serbia	88.0
15	Sanditi	Netherlands	83.0
16	Alpha	Netherlands	94.0
17	Plato	Germany	85.0
18	Niva	Czech Republic	94.0
RC[1]	Arone (*A. elatius*)		54
	Gondolin (*F. rubra*)		89
	Amba (*D. glomerata*)		83
	Pimpernell (*L. perenne*)		90

RC= Reference crop; 1= The grass mixture consists of 25% of each of the mentioned species.

Iranian alfalfa ecotypes have been divided into 6 commercial groups depending on their production area, of which one group is located in middle and west, one group in northwest, two groups in east, one group in southeast and one group in southwest of Iran. The ecotypes used in this study are belonging to two groups of ecotypes from west and northwest of Iran. Both trials have been hand seeded in May, 2006. The first experimental year was considered as the establishment year. During the establishment, plots were hand clipped one time in September 2006. To estimate BNF, nine field plots in each experiment were hand seeded with a mixture of four grasses as reference crop (Table 2) so that the reference crop was on the first and the last plot of each incomplete block. The grass-mixture consisted of tall oat-grass (*Arrhenatherum elatius* var**.** Arone), red fescue (*Festuca rubra* var. Gondolin), cocksfoot-grass (*Dactylis glomerata* var. Amba) and perennial ryegrass (*Lolium perenne* var. Pimpernell). The seeding density was 25 kg ha^{-1} in all cases, adjusted by the germination rate of the cultivars.

The field plots, in both experiments, were laid out in an α-lattice design with two complete blocks or replications. Each replication consisted of three incomplete blocks and each incomplete block consisted of six experimental alfalfa plots that were surrounded by one reference crop plot at each side. All cultivars were present in each complete block or replication. Each genotype was seeded in 12 rows with 1.5-m long in rain-fed trial at Raasdorf and 8 rows with 1-m long in irrigated trial at Gross-Enzersdorf. Spacing between rows in both trials was 12.5 cm. In both experiments, nine plots of reference crop were seeded similar to alfalfa plots. In irrigated trial, soil moisture content was monitored weekly by four FDR (Frequency Domain Reflectometry) probes in 15, 40, 80 and 120 cm soil depths; these devices were installed in one plot in each incomplete block including cultivars of 1, 9 and 18 in one replication. Irrigation was started at 50 % depletion of soil available water (SAW) content (SAW = Water content difference between field capacity and permanent wilting point) based on FDR probe in 15cm soil depth. The amount of applied irrigation water was calculated for 0-30cm depth based on soil moisture content up to field capacity. Plots were irrigated by a drip irrigation system. There were 28 drippers per plot with 2 litres water outflow per hour and dripper.Site classification was mainly related to level of summer drought stress (irrigated and rain-fed cropping) and different water holding capacity of locations (depth of A horizon and organic matter content of soil).

Data collection

Plots were hand clipped three times at 30-40 % of flowering using a garden scissor to a 5-cm stubble height on 1-2 June, 1-2 August and 20-21 September in irrigated trial and 7-8 June, 11-12 August and 29-30 September in rain-fed in 2007 ; 5-6 June ,19-20 July and 15-16 September in irrigated and 11-12 June, 28-29 July and 29-30 September in rain-fed in 2008. All traits were measured at harvesting time. Root dry matter, stubble dry matter and inorganic nitrogen in 30-60 cm and 60-90 cm soil depths were recorded only at the third harvest in each year. Shoot (SHDM) and stubble (STDM) yield data (t ha^{-1}) were adjusted to a dry matter basis by sub-sampling approximately 200 and 50 g of fresh shoot and stubble, respectively, from 0.5 m^2 of the plots at each harvest, and drying the samples at 60 °C for 48 h. Annual shoot and stubble dry matter production was determined by summing the yield data over the harvests within each year. Root dry matter (RODM) (t ha^{-1}) was determined using a soil corer with 9 cm diameter. Two samples (one between and one on the row) were taken in each plot down to 30 cm depth and fresh root after washing was dried at 60°C for 48 h. Crop regrowth (cm) (CR) was measured 18-20 days after each harvest based on the average of plant height (cm) in 3 points per plot. Plant height (cm) (PH), number of stems per m^2 (STN), number of nodes per stem (NN), leaf to stem ratio (LSR) and Leaf area index (LAI) were measured at harvest time each year and the average of harvests in each year was used in data analysis. Number of stems per m^2 (STN) and leaf to stem ratio (LSR) were determined in a sub-sample of 0.25 m^2 in each plot. Number of nodes per stem (NN) was the average of the number of nodes in five randomly selected stems per plot. Leaf area index (LAI) was measured using

LAI-2000 Plant Canopy Analyzer (LI-COR, Lincoln, NE), before each harvest. Chlorophyll content (mg m^{-2} leaves) was measured using a portable chlorophyll meter, Yara N-tester (Yara international ASA ,Norway, www.yara.com) at harvesting time each year. The average of 30 reading of the most fully expanded leaves in the upper 15 cm of the canopy was used as plot value.

Biological Nitrogen Fixation (BNF) was estimated by the “extended difference method” for each plot (Giller 2001). Based on this method, the BNF of the legume crop was taken as the difference between the total N uptake of the legume and that of the non-nodulating plant (reference crop), where both were grown at the same time on the same field regarding differences in soil inorganic N contents between the plots as well. Plant samples were taken at the harvesting time. Nitrogen content was determined in dry plant organs with an isotope ratio mass spectrometer (IRMS-ThermoQuest Finnigan DELTAplus) in the laboratory of the Department of Chemical Ecology, University of Vienna. According to this method Biological Nitrogen Fixation Capacity (BNF [kg ha^{-1}]) is calculated with the data from the plant analyses (without regarding crop litter N) as follows:

$$\text{BNF [kg ha}^{-1}] = (\text{Shoot N}_{\text{Leg}} + \text{Stubble N}_{\text{Leg}} + \text{Root N}_{\text{Leg}} \text{ [kg ha}^{-1}]) - (\text{Shoot N}_{\text{Ref}} + \text{Stubble N}_{\text{Ref}} + \text{Root N}_{\text{Ref}} \text{ [kg ha}^{-1}]) + (\text{N}_{\text{in}} \text{ in soil}_{\text{Leg}} - \text{N}_{\text{in}} \text{ in soil}_{\text{Ref}} \text{ [kg ha}^{-1}])$$

Where Shoot N_{Leg}, Stubble N_{Leg} and Root N_{Leg} are nitrogen content in alfalfa shoot, stubble and root; Shoot N_{Ref} , Stubble N_{Ref} and Root N_{Ref} are nitrogen content in shoot, stubble and root of reference crop (Grass mixtures); N_{in} in $soil_{Leg}$ and N_{in} in $soil_{Ref}$ are inorganic soil nitrogen contents under alfalfa and reference crop, respectively.

Protein content (CP) based on dry matter of different plant parts (shoot, stubble and root) was calculated by multiplying N content of plant parts by a factor of 6.25.

Statistical analysis

Analysis of variance and mean comparisons

The data were analyzed based on repeated measure analysis of variance based on an alpha-lattice design. A linear mixed model was used, where location (L), Replication (Rep) and genotype (G) were considered as fixed effects, while incomplete block within replication [iblock (rep)] and year (Y) were considered as random effects and repeated measure, respectively. The analysis was done using two different covariance structures, the unstructured (UN) and the first-order autoregressive AR (1). The Akaike Information Criterion (AIC) was used to find the best model describing the covariance structure. The data were analyzed by PROC MIXED in SAS software (SAS Institute, 2004). Denominator degrees of freedom (DDF) were calculated using the Kenward-Roger (KR) method. Difference between Iranian ecotypes and European cultivars in measured traits was evaluated by contrast using CONTRAST statement in SAS software (SAS Institute, 2004). Least square (LS) means were estimated for locations, cultivars and cultivar by location interactions. Mean comparisons were adjusted for the *p*-values (α =0.05) using ADJUST=SIMULATION option in SAS software. Regarding genotype by location interaction (GL), LS-mean comparisons were carried out among cultivars in each location. A SAS macro was used to find a letters display for all pairwise mean comparisons (Piepho, 2009)

Partitioning of genotype by environment interaction (GEI) and stability analysis

Combination of years and locations were considered as environments which resulted in a total of 4 environments. Adjusted LS-mean of genotypes for each environment produced by mixed model was used to construct the genotype by environment (GE) two-way table.

Assessments of the stability as well as the performance of plant genotypes across diverse environmental conditions are important to plant breeders and agronomists as tools for selecting superior cultivars for the target environments. The parameter of coefficient of variability (CV_i) (Francis and Kannenberg 1978), stability variance ($б_i^2$) (Shukla 1972), superiority statistic (P_i) (Lin and Binns 1988) were calculated as univariate stability statistics. These parameters were calculated using Excel spread sheets as follows:

$$P_i = \frac{\sum_{j=1}^{q} (X_{ij} - M_j)^2}{2q} ,$$

$$\sigma_i^2 = \left[\frac{p}{(p-2)(q-1)} \sum_{j=1}^{q} (X_{ij} - \overline{X}_{i.} - \overline{X}_{.j} + \overline{X}_{..})^2 \right] - \frac{\sum_{i=1}^{p} \sum_{i=1}^{q} (X_{ij} - \overline{X}_{i.} - \overline{X}_{.j} + \overline{X}_{..})^2}{(p-1)(p-2)(q-1)}$$

$$CV_i = 100 \left(S_i \big/ \overline{X}_{i.} \right) \quad , \qquad S_i^2 = \frac{\sum_{j=1}^{q} (X_{ij} - \overline{X}_{i.})^2}{q-1}$$

Where X_{ij} is the yield of genotype i in environment j , $\overline{X}_{i.}$ the mean yield of genotype i over environments, $\overline{X}_{.j}$ the mean yield of environment j over genotypes, $\overline{X}_{..}$ the grand mean, p number of genotypes, q number of environment and S_i^2 the variance of genotype i across environments.

The additive main effects and multiplicative interaction (AMMI) analysis was performed as multivariate GEI analysis. GE_{ij} interaction effects for shoot DM, total biomass yield and BNF were partitioned by AMMI analysis using the software IRRISTAT (version 5.02) into:

$$GE_{ij} = X_{ij} - \overline{X}_{i.} - \overline{X}_{.j} + \mu = \sum_n \lambda_n \gamma_{in} \delta_{jn} + R_{ij}$$

where GE_{ij} is interaction effect for genotype i in environment j, X_{ij} the yield of genotype i in environment j, μ the grand mean, $\overline{X}_{i.}$ the mean yield of genotype i over environments, $\overline{X}_{.j}$ the mean yield of environment j over genotypes, λ_n the singular value for interaction principal component axis *n*(IPCA), γ_{in} eigenvector value for genotype *i* and IPCA axis *n*, δ_{jn} eigenvector value for environment *j* and IPCA axis *n* and R_{ij} the residual term.

The IPCA1 scores for both genotypes and environments were plotted in first biplot (AMMI1) against the trait means for the genotypes and environments, respectively. The scores of genotypes and environment on the first two IPCA axes were jointly represented in a second biplot (AMMI2 biplot).

AMMI's stability value (ASV) was calculated as suggested by Purchase (1997). The ASV is the distance from the coordinate point to the origin in a two dimensional of IPCA1 scores against IPCA2 scores in the AMMI model (Purchase *et al.*, 2000*).* Because the IPCA1 score contributes more to the GE interaction sum of square (SS), a weighted value is needed. This weight is calculated for each genotype and each environment according to the relative contribution of IPCA1 to IPCA2 to the interaction SS as follows:

$$ASV_i = \sqrt{\left[\frac{SS_{(IPCA\ 1)}}{SS_{(IPCA\ 2)}} (IPCA\ 1 score) \right]^2 + (IPCA\ 2 score)^2}$$

where SS $_{(IPCA1)}$ and $SS_{(IPCA2)}$ are sum of squares of first and second interaction principal component axis, and the ratio of $SS_{(IPCA1)}/SS_{(IPCA2)}$ is the weight given to the IPCAl scores. Simple coefficient of correlation was computed for each pair of the stability parameters by SPSS software (version 15).. Low value of all stability parameters calculated in this study are regarded as being stable and desired. The genotype with the best yield or stability received rank 1. For selecting and recommendation purpose of genotypes based on all three characters and four stability parameters, the ranks of a genotype in all situations were added up together and the genotype with the lowest value was selected as the best one.

Simple correlations and path analysis

Phenotypic correlations between traits based on adjusted LS-mean of genotypes across years (n=18) were calculated for each condition. Also, the relative importance of direct and indirect effects of characters (causal variables) on BNF, shoot DM and root DM (as effect or dependent variables) were determined in a set of structural models by path analysis for each condition. Standardized partial regression coefficients from the regression analysis were used as path coefficients for the respective predictor variables. The indirect effects were determined by multiplying the correlation by their respective path coefficients. In the path analysis, correlation coefficient between a causal variable and an effect (dependent) variable is decomposed into the direct effect of the casual variable per se, and indirect effects via other casual variables in the model on effect variable:

$$r_{iy} = P_{iy} + \sum_j r_{ij} P_{jy}$$

where r_{iy} and r_{ij} are the correlations between casual character i and effect character y and the correlation between casual characters i and j, respectively; P_{iy} and P_{jy} are path coefficients of the casual characters i and j (the direct effects of character i and j on character y). The part of $\sum_j r_{ij} P_{jy}$ in the above formula is the sum of indirect effects of casual variable i on dependent variable y via other casual variables in the model. The residual in each model was computed as follow:

$$\text{Residual} = \sqrt{1 - R^2} \ ,$$

where R^2 is the coefficient of determination of the model. Correlation and path (standardized partial regression coefficient) coefficients were computed by the software SPSS (version 15) and indirect effects by Excel spread sheet program. The result of path analysis was displayed diagrammatically as a path diagram, for each condition.

Cluster analysis

Hierarchical cluster analysis was used to group and assess the suitability of genotypes in each condition using adjusted mean values of morphological data in addition to shoot dry matter, shoot protein content and root dry matter. Additionally, genetic distance and divergence was measured between genotypes based on mentioned characters. The analysis adopted the Ward method and an Euclidean distance as the dissimilarity measure between genotypes (Crossa *et al.*, 1995). Data were standardized by transforming values to Z scores for each character before analysis:

$$Z = \frac{x - \mu}{\sigma}$$

where x is a raw value to be standardized; μ is the mean of the character; and σ is the standard deviation of the character. The analysis was done by SPSS software (version 15).

Results

One of our main goals for this study was to monitor forage yield and BNF potential among tested cultivars and ecotypes under no water stress and natural (rainfall) management conditions in organic farming system. Thus, irrigation scheduling in the irrigated trial was aimed to prevent moisture stress among genotypes whereas the rain-fed one was not irrigated similar to organic farmers' management in eastern Austria. Temperature patterns were similar across the two years and locations while precipitation distributions were different between the two years but similar for the two locations (Fig. 1). The amount of precipitation was nearly doubled during June to August 2008 compared to 2007. So, the genotypes in rain-fed trial were imposed to mid-season water stress during 2007 and late-season stress during 2008.The difference between the two locations concerning organic carbon content and depth of the A

Table 3. Significance level for the fixed effects and their interactions on the studied traits.

Trait Effect	CR	PH	STN	NN	CHL	LAI	LSR	SH-DM	ST-DM	RO-DM	TBY	BNF	SCP	RCP
Location(L)	***	***	***	ns	***	***	**	***	ns	**	***	***	ns	ns
Year(Y)	***	***	***	***	***	***	***	***	ns	**	***	***	***	***
Genotype(G)	**	***	***	ns	ns	***	***	***	*	***	***	***	***	*
Iranian vs. European	*	**	***	**	ns	***	***	***	**	***	*	***	***	***
L*Y	**	ns	**	***	**	***	*	**	ns	ns	**	**	***	ns
G*L	**	*	***	*	ns	ns	ns	***	**	***	***	***	**	ns
G*Y	*	*	***	ns	ns	ns	ns	*	ns	***	**	*	**	ns
GLY	**	*	***	ns	ns	ns	ns	***	ns	ns	***	*	ns	ns

CR=Crop regrowth; PH= Plant height; STN= Stem number per m^2; NN= Node number; CHL= Chlorophyll content; LAI= Leaf area index; LSR= Leaf to stem ratio; SHDM, STDM and RODM= Shoot, stubble and root dry matter, respectively; TBS= Total biomass yield; BNF= Biological nitrogen fixation; SCP and RCP = shoot and root crude protein, respectively. The significance level ρ<0.001, 0.001<ρ< 0.01, 0.01<ρ< 0.05 and not significance are indicated with ***, **,* and ns, respectively.

horizon of soil (Table 1) result in a better water-holding capacity in the irrigated than the rain-fed trial. The second and third harvests were affected by water stress in 2007 while only third harvest was affected in 2008. Generally, we considered irrigated trial as normal and rain-fed one as water stress conditions.

Analysis of variance and mean Comparisons

The significance levels of fixed effects are presented based on repeated measure analysis of variance using mixed model in Table 3 for different traits. The appropriate covariance structure to analyze each trait, selected by Akaike Information Criterion (AIC), is shown in Table 4. The unstructured (UN) covariance structure has been applied for crop regrowth, stem

Table 4. Selected covariance structure based on the value for the Akaike Information Criterion (AIC) in the analysis of variance for studied traits.

Trait	CR	PH	STN	NN	CHL	LAI	LSR	SH-DM	ST-DM	RO-DM	TBS	BNF	SCP	RCP
Covariance structure	UN	AR(1)	UN	AR(1)	UN	AR(1)	UN	AR(1)	UN	UN	AR(1)	AR(1)	UN	UN

UN = Unstructured; AR (1) = First order autoregressive; See Table 3 for abbreviations.

number per m^2, chlorophyll content, leaf to stem ratio, stubble dry matter, root dry matter, shoot and root protein content. The first order autoregressive has been used for other characters. Because of 2 levels for repeated measure factor (year), the results of the first-order autoregressive [AR (1)] will be identical to compound symmetry (CS).

The location main effect, i.e. irrigated and rain-fed conditions, was significant on crop regrowth, plant height, stem number per m^2, chlorophyll content, LAI, shoot dry matter, total biomass yield, leaf to stem ratio, root dry matter and BNF. Except for leaf to stem ratio, shoot and root protein content, average of all traits were reduced under stress condition in rain-fed trial (Table 5 and Fig. 2). LAI, shoot dry matter, crop regrowth, total biomass yield, plant height and BNF showed the highest reduction with 42.7, 36.1, 34.5, 28.6, 26.5 and 24.5 %, respectively. The lowest reduction was found for chlorophyll content (- 3.6 %) and node number per stem (- 4.5 %). Leaf to stem ratio, shoot and root protein content have been increased by 18.6, 0.9 and 2.9 % under rain-fed condition, respectively.

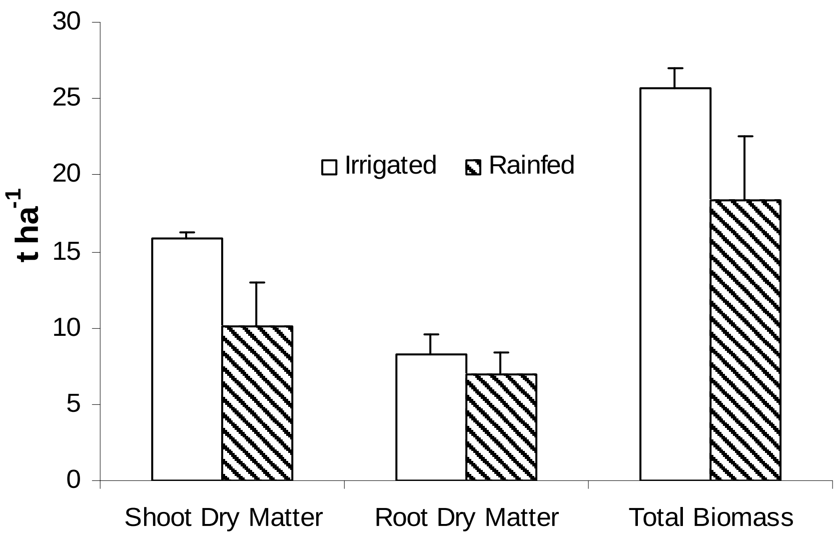
The year main effect was significant, at different probability levels, on all measured traits except for stubble dry matter. These annual differences of traits, especially in rain-fed trial, reflect mainly different distribution of precipitation (Fig. 1) during 2007 and 2008. Regarding the suitable distribution of rainfall during the growing season in 2008, especially between June and August (Fig. 1), performance of genotypes was better in 2008 than in 2007 and intensity of drought stress under rain-fed condition based on biomass production was less than that of 2007.

The genotype main effect was significant for all traits except for node number per stem and chlorophyll content (Table 3). The significant results for genotypic effects in different traits exhibit suitable genetic variation among tested genotypes.

Table 5. LS-mean estimates and standard errors (SE) of traits for two conditions.

Trait Condition	CR	PH	STN	NN	CHL	LAI	LSR	SH-DM	ST-DM	RO-DM	TBS	BNF	SCP	RCP
	cm	cm			mg/m2			t h^{-1}	t h^{-1}	t h^{-1}	t h^{-1}	kg h^{-1}	%	%
Irrigated	28.6	86.2	1101.6	10.9	693.4	4.4	0.7	15.9	1.5	8.3	25.6	404	22.6	15.8
Rain-fed	18.7	63.3	956.2	10.4	668.8	2.5	0.8	10.1	1.2	6.9	18.3	305	22.8	16.3
Stress effect (%)	-34.5	-26.5	-13.2	-4.5	-3.6	-42.7	18.6	-36.1	-15.5	-16.3	-28.6	-24.5	0.9	2.9
SE	0.3	0.7	7.2	0.2	3.7	0.03	0.02	0.4	0.1	0.2	0.5	18.8	0.1	0.2

See Table 3 for abbreviations.



Error bar shows one standard deviation.

Figure 2. Average of shoot and root dry matter (t ha^{-1}) and total biomass yield (kg ha^{-1}) under two different conditions.

Least square (LS) means of genotypes over years and locations are shown in Table 6. NS-Banat, Shorakat and Khorvande had the most rapid regrowth after cutting which was significantly greater than that of Plato ZS. Although the ecotype Khorvande had a rapid regrowth after cutting, its height at cutting time was the shortest. Ghara-aghaj (78.6 cm) was the tallest which showed significant difference with Khorvande (68.4 cm) as the shortest genotype. Ghara-aghaj and Niva with 1147.2 and 946.3 had the highest and lowest stem number per m^2. Despite of the greatest plant height and stem number per m^2 of the ecotype Ghara-aghaj, it did not show the highest shoot dry matter and total biomass yield,

Table 6. LS-mean estimates and standard errors (SE) for genotypes over years and locations.

Trait	CR		PH		STN		NN		CHL		LAI		LSR	
Genotype	cm		cm						mg m^{-2}					
Mohajeran	24.7	ab	75.1	bc	1108.6	def	10.4	a	681.3	a	3.3	ab	0.7	abc
Khorvande	25.3	bc	68.4	a	955.8	ac	10.8	a	670.4	a	3.0	a	0.8	cef
Famenin	24.3	ab	74.8	bc	1069.2	cf	10.1	a	671.3	a	3.4	ab	0.7	a
Gharghologh	22.7	ab	72.7	ab	1085.1	bef	10.3	a	695.6	a	3.2	ac	0.7	af
Ordobad	23.2	ab	71.6	ac	990.4	abcd	10.0	a	683.7	a	3.3	ab	0.7	acd
Shorakat	25.6	bc	75.8	bc	1117.3	ef	10.6	a	676.0	a	3.4	ab	0.7	ae
Ghara-aghaj	24.5	ab	78.6	b	1147.2	f	10.5	a	689.7	a	3.5	bc	0.7	ac
Hokm-abad	23.4	ab	73.8	ab	1067.5	af	10.8	a	683.6	a	3.3	ab	0.7	acd
Sitel	23.9	ab	75.9	bc	979.8	abc	10.6	a	705.6	a	3.6	bc	0.8	bdf
Verko	22.1	ab	73.7	ab	1002.9	ace	10.4	a	691.0	a	3.5	bc	0.9	df
Vlasta	24.3	ab	77.2	bc	1060.6	cf	10.5	a	679.4	a	3.7	bc	0.8	af
Monz 42	21.7	ac	76.5	bc	987.1	abcd	10.8	a	676.1	a	3.8	b	0.8	cf
Fix 232	23.6	ab	76.2	bc	982.4	abc	10.8	a	656.0	a	3.6	bc	0.9	f
NS_Banat	25.9	b	74.4	ab	972.2	abc	10.8	a	671.2	a	3.5	bc	0.8	cef
Sanditi	24.5	ab	77.7	bc	1009.0	ace	11.0	a	687.9	a	3.7	bc	0.8	af
Alpha	22.1	ab	73.0	ab	1066.5	cf	10.5	a	667.8	a	3.5	bc	0.8	cf
Plato ZS	20.6	a	74.1	ab	972.5	abc	11.2	a	690.2	a	3.7	bc	0.8	cef
Niva	23.8	ab	76.4	bc	946.3	c	11.2	a	683.2	a	3.4	ab	0.8	af
SE	0.8		1.2		22.1		0.3		11.2		0.09		0.03	
Iranian	24.2		73.9		1068		10.4		681.5		3.3		0.72	
European	23.3		75.5		997.9		10.8		680.8		3.6		0.83	

Continued Table 6.

Trait	SHDM		STDM		RODM		TBS		BNF		SCP		RCP	
Genotype	t ha^{-1}		t ha^{-1}		t ha^{-1}		t ha^{-1}		Kg ha^{-1}		%		%	
Mohajeran	13.9	de	1.3	a	7.4	bce	22.8	cd	378	bcdfgh	22.8	bc	16.2	ab
Khorvande	9.6	a	1.4	a	8.5	def	19.5	a	293	a	22.8	bc	17.7	b
Famenin	12.9	bcd	1.2	a	7.3	ae	21.4	ac	312	ab	21.4	a	16.7	ab
Gharghologh	11.7	bc	1.3	a	9.3	f	22.5	bcd	349	ah	22.2	ab	16.2	ab
Ordobad	11.7	bc	1.3	a	8.1	cef	21.2	ac	325	ag	22.8	bc	16.6	ab
Shorakat	13.1	be	1.4	a	8.7	ef	23.0	cd	369	bcdfgh	22.3	ab	15.7	ab
Ghara-aghaj	13.5	ce	1.2	a	7.1	acd	21.9	ac	337	ah	22.0	ab	16.5	ab
Hokm-abad	11.4	ab	1.2	a	8.5	def	21.1	ac	328	af	22.8	bc	16.8	ab
Sitel	14.9	e	1.5	a	7.3	ae	23.6	c	389	cfgh	22.4	ab	15.3	a
Verko	13.6	ce	1.4	a	7.0	acd	21.8	ac	367	bcdfgh	23.0	bc	15.8	ab
Vlasta	14.0	de	1.3	a	8.0	bcef	23.4	cd	395	fgh	23.2	bc	15.3	a
Monz 42	12.3	bcd	1.5	a	7.3	ae	21.0	ad	320	ad	22.8	bc	15.7	ab
Fix 232	13.9	de	1.4	a	8.0	bcef	23.2	cd	398	eh	22.8	bc	15.8	ab
NS_Banat	13.5	ce	1.5	a	8.5	def	23.5	c	407	h	22.7	ac	16.2	ab
Sanditi	13.5	ce	1.3	a	6.5	ab	21.3	ac	338	aef	23.1	bc	15.2	a
Alpha	13.1	be	1.3	a	5.8	a	20.3	ab	318	ac	23.2	bc	15.7	ab
Plato ZS	13.9	de	1.6	a	6.5	ab	22.1	bcd	392	dfh	24.0	c	15.7	ab
Niva	13.8	de	1.3	a	6.8	ac	21.9	ac	368	bcdfgh	23.2	bc	16.3	ab
SE	0.4		0.1		0.3		0.6		18.4		0.3		0.4	
Iranian	12.2		1.3		8.1		21.7		336		22		16.6	
European	13.6		1.4		7.2		22.2		369		23		15.7	

See Table 3 for abbreviations.

probably due to competition among the high number of shoots in low input organic farming system. Sitel, Vlasta, Plato ZS and Mohajeran had the highest shoot dry matter yield with 14.85, 14.04, 13.93 and 13.92 t h^{-1}, respectively. Ghargologh, Shorakat, NS-Banat, Khorvande, and Hokmabad had the highest root DM yields. It seems that the rapid regrowth of NS-Banat, Shorakat and Khorvande is related to their high root biomass. The BNF estimations of NS-Banat, Fix232, Vlasta and PlatoZS were the greatest with 407, 398, 395 and 392 kg ha^{-1}, respectively.

The difference between Iranian ecotypes and European cultivars was tested by a contrast equation. The result of contrasts for all traits except chlorophyll content was significant (Table 3).

The interaction between genotype and location (GL) was significant for crop regrowth, plant height, stem number per m^2 , node number per stem, shoot dry matter, stubble dry matter, root dry matter, total biomass, BNF, shoot protein content (Table 3).

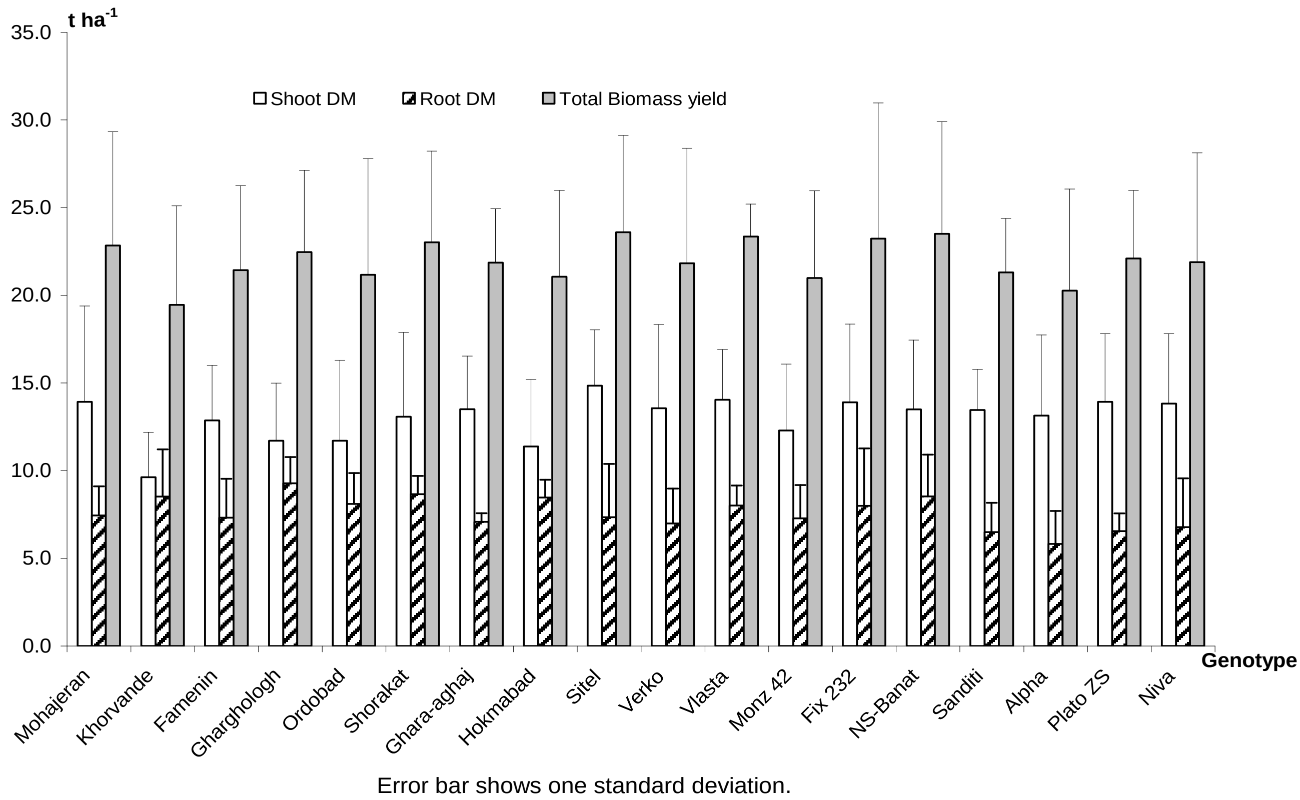


Fig. 3. Shoot and root dry matter (t ha^{-1}) and total biomass yield (t ha^{-1}) of genotypes over years and locations.

Table 7. LS-mean of genotypes for some traits in each location over years.

Cultivar	PH (cm) IR		RN		STN (m^{-2}) IR		RN		LSR IR		RN		SCP % IR		RN	
Mohajeran	88.8	b	61.3	ab	1183.6	bcd	1033.6	bcd	0.57	a	0.85	ab	21.8	ab	23.8	cd
Khorvande	77.5	a	59.2	ab	1016.6	ac	895.1	ac	0.71	ab	0.96	b	22.5	ac	23.1	ac
Famenin	86.5	ab	63.2	ab	1148.0	ad	990.5	bcd	0.61	ab	0.73	a	21.4	a	21.3	a
Ghargologh	85.1	ab	60.4	ab	1100.7	ad	1069.4	cd	0.67	ab	0.80	ab	22.3	ac	22.2	ac
Ordobad	84.8	ab	58.5	a	1207.6	cd	773.2	a	0.66	ab	0.77	ab	22.7	ac	22.9	ac
Shorakat	89.8	b	61.8	ab	1234.7	d	999.8	bcd	0.63	ab	0.76	ab	22.2	ac	22.5	ac
Ghara-aghaj	89.1	b	68.1	b	1193.4	cd	1101.0	d	0.64	ab	0.76	ab	22.5	ac	21.5	ab
Hokmabad	84.4	ab	63.3	ab	1152.4	ad	982.5	bcd	0.63	ab	0.80	ab	23.1	ac	22.5	ac
Sitel	86.0	ab	65.8	ab	1005.0	ab	954.5	ad	0.79	ab	0.90	ab	22.9	ac	21.9	ad
Verko	86.8	ab	60.5	ab	1130.7	ad	875.1	ac	0.79	ab	0.92	ab	23.1	ac	22.9	ac
Vlasta	86.5	ab	67.9	ab	1118.3	ad	1003.0	bcd	0.76	ab	0.84	ab	23.5	bc	23.0	ac
Monz 42	89.9	b	63.0	ab	1042.3	ac	931.8	ad	0.78	ab	0.90	ab	22.8	ac	22.8	ac
Fix 232	87.2	b	65.3	ab	1080.4	ad	884.4	ac	0.80	ab	0.93	ab	22.2	ac	23.5	bcd
NS-Banat	84.1	ab	64.7	ab	987.3	a	957.2	ad	0.78	ab	0.87	ab	22.8	ac	22.6	ac
Sanditi	89.1	b	66.3	ab	1024.6	ac	993.4	bcd	0.76	ab	0.85	ab	22.8	ac	23.5	bcd
Alpha	85.7	ab	60.3	ab	1094.3	ad	1038.7	bcd	0.78	ab	0.90	ab	22.3	ac	24.0	c
Plato ZS	84.1	ab	64.2	ab	1082.1	ad	862.9	ab	0.79	b	0.87	ab	24.0	c	24.0	c
Niva	86.5	b	66.3	ab	1027.4	ac	865.2	ab	0.73	ab	0.86	ab	22.9	ac	23.6	cd
SE	1.70				31.23				0.04				0.35			

Table 7. Continued

Cultivar	SHDM (t ha^{-1}) IR		RN		RODM (t ha^{-1}) IR		RN		TBS (t ha^{-1}) IR		RN		BNF (kg ha^{-1}) IR		RN	
Mohajeran	18.45	e	9.40	ad	7.53	abc	7.37	ac	27.83	cde	17.83	ab	458.7	cde	297.9	ab
Khorvande	11.45	a	7.79	a	10.21	d	6.83	ac	23.26	ab	15.66	a	336.3	b	249.4	a
Famenin	15.46	bcd	10.27	ad	8.41	ad	6.23	a	25.01	bc	17.85	ab	349.7	ab	273.7	a
Ghargologh	13.63	ab	9.79	ad	10.46	d	8.09	ac	25.63	be	19.32	ac	387.1	bd	310.3	ab
Ordobad	15.14	bcd	8.26	ab	9.40	cd	6.80	ac	26.11	be	16.22	ab	395.5	be	254.2	a
Shorakat	16.34	bce	9.81	ad	8.36	ad	8.97	c	26.18	be	19.87	bc	404.0	be	333.6	ab
Ghara-aghaj	15.53	bcd	11.49	cd	6.92	ab	7.23	ac	23.80	ab	19.93	bc	339.7	b	334.3	ab
Hokmabad	14.34	ac	8.43	ac	9.23	bd	7.70	ac	24.99	bc	17.13	ab	404.6	be	252.3	a
Sitel	17.40	de	12.30	d	8.18	ad	6.50	ab	27.09	ace	20.11	bc	435.0	be	343.2	ab
Verko	17.36	ce	9.75	ad	8.13	ad	5.86	a	26.74	be	16.92	ab	447.8	be	285.6	ab
Vlasta	16.40	bce	11.68	de	7.16	abc	8.86	bc	24.79	bc	21.91	c	405.2	be	385.2	b
Monz 42	15.49	bce	9.09	ace	8.15	ad	6.39	ab	24.98	bc	17.01	ab	382.8	bd	257.1	a
Fix 232	17.23	de	10.57	bcd	10.44	d	5.54	a	29.23	e	17.22	ab	495.6	e	301.4	ab
NS-Banat	16.10	bce	10.88	ad	10.58	d	6.49	ab	28.37	ce	18.63	ac	485.1	de	328.8	ab
Sanditi	15.28	bcd	11.63	de	6.23	a	6.77	ac	22.97	b	19.63	ac	340.7	ab	335.5	ab
Alpha	16.81	ce	9.47	ad	5.94	a	5.70	a	24.02	abd	16.51	ab	352.6	bc	283.3	ab
Plato ZS	16.98	ce	10.87	bcd	6.44	a	6.66	ac	25.25	bc	18.95	ac	442.2	be	341.4	ab
Niva	16.54	ce	11.10	bcd	7.04	abc	6.52	ac	25.00	bc	18.77	ac	411.5	be	325.2	ab
SE	0.62				0.44				0.85				26.04			

IR=Irrigated; RN=rain-fed. See table 3 for abbreviation. Values in a column with the same letter do not differ significantly ($p < 0.05$).

Also, significant interaction between year and genotype (GY) was detected for crop regrowth, plant height, stem number per m^2, shoot dry matter, root dry matter, total biomass yield, BNF, dry root mass density, shoot to root ratio, and shoot protein content (Table 3). The significant result for genotype by environment (GL, GY and GLY in Table 3) interaction, especially for biomass production and BNF, lead us to consider production stability of genotypes as well as a high performance.

Stability analysis

Stability analysis was performed for shoot dry matter (t ha^{-1}), total biomass yield (t ha^{-1}) and biological nitrogen fixation (BNF) (kg ha^{-1}) by calculating of 4 stability parameters (Tables 8a, b, c and 10).

Univariate stability statistics

Based on classification of stability parameters by Lin *et al*. (1986), coefficient of variability (CV_i) was classified in type 1 stability group, which is analogous to the homeostasis (Lin *et al*., 1986) , biological (Baker 1981) or static (Leon 1985) concept of stability. With regard to this concept, a stable genotype possesses an unchanged performance regardless of any variation of the environment (Becker and Leon 1988). Based on CV_i, concerning shoot dry matter, Sanditi, Vlasta, Sitel, Ghara-aghaj and Famenin were the most stable genotypes (Table 8a). Mohajeran, Ordobad, Shorakat and Verko had the highest CV_i values and were the least stable based on this measure. CV_i was differently correlated to shoot dry matter yield under irrigated, rain-fed and average across the two conditions (Table 11). A positive correlation (r = 0.22) was found between CV_i and shoot dry matter under irrigated condition, whereas the correlation was negative under rain-fed condition (r = -0.68**) and for the average over the two conditions (r = -0.20). This shows that in no water stress condition high yielding genotypes may have high variability, while in water stress condition the variability of high yielding genotypes was low. Mohajeran, Verko and Fix232 were the genotypes with high shoot dry matter under irrigated condition (Table 7) which showed high CV_i (Table 8a), and Sitel, Vlasta, Sanditi and Ghara-aghaj with high shoot dry matter under rain-fed conditions (Table 7) showed low CV_i (Table 8a). Type 1 stability is often associated with a relatively poor response and low yield in environments that are high yielding for other cultivars (Lin *et al*., 1986).With regard to average shoot dry matter over 2 conditions and CV_i values, Sitel, Vlasta and Ghara-aghaj can be selected with high and stable performance.

Table 8. Shoot dry matter (a), total biomass yield (b) and BNF (c) of genotypes along with their stability values and ranks based on CV_i, $б_i^2$ and P_i.

(a)	Shoot DM ($t\ ha^{-1}$)		Stability parameters					
			CV_i		$б_i^2$		P_i	
Genotype	Mean	**Rank**	Value	**Rank**	Value	**Rank**	Value	**Rank**
Mohajeran	13.92	**4**	39.3	**18**	5.5	**18**	2.4	**5**
Khorvande	9.62	**18**	26.7	**6**	1.9	**12**	19.7	**18**
Famenin	12.86	**13**	24.4	**5**	1.9	**13**	5.1	**13**
Gharghologh	11.71	**15**	28.0	**8**	2.2	**14**	9.8	**17**
Ordobad	11.70	**16**	39.2	**17**	1.3	**10**	8.2	**15**
Shorakat	13.08	**12**	36.8	**16**	3.5	**17**	4.3	**11**
Ghara-aghaj	13.51	**8**	22.4	**4**	2.5	**16**	4.4	**12**
Hokm-abad	11.38	**17**	33.5	**13**	0.0	**1**	9.3	**16**
Sitel	14.85	**1**	21.3	**3**	0.3	**2**	0.8	**1**
Verko	13.55	**7**	35.3	**15**	1.3	**9**	2.7	**6**
Vlasta	14.04	**2**	20.4	**2**	1.3	**8**	2.2	**4**
Monz 42	12.29	**14**	30.9	**11**	0.8	**4**	5.9	**14**
Fix 232	13.90	**5**	32.0	**12**	0.8	**5**	1.9	**3**
NS_Banat	13.49	**9**	29.3	**10**	1.1	**7**	3.2	**7**
Sanditi	13.45	**10**	17.2	**1**	2.2	**15**	3.8	**9**
Alpha	13.14	**11**	35.0	**14**	1.1	**6**	3.9	**10**
Plato ZS	13.93	**3**	27.9	**7**	0.5	**3**	1.5	**2**
Niva	13.82	**6**	28.9	**9**	1.5	**11**	3.4	**8**
Mean	13.0		29.4		1.6		5.1	

(b)	Total biomass yield ($t\ ha^{-1}$)		Stability parameters					
			CV_i		$б_i^2$		P_i	
Genotype	Mean	**Rank**	Value	**Rank**	Value	**Rank**	Value	**Rank**
Mohajeran	22.83	**6**	28.5	**12**	5.7	**12**	8.9	**6**
Khorvande	19.46	**18**	29.0	**15**	1.4	**4**	24.7	**18**
Famenin	21.43	**12**	22.5	**6**	0.6	**3**	11.8	**10**
Gharghologh	22.47	**7**	20.7	**5**	0.2	**1**	8.1	**4**
Ordobad	21.16	**14**	31.4	**17**	5.8	**13**	16.4	**16**
Shorakat	23.02	**5**	22.6	**7**	10.2	**16**	9.3	**7**
Ghara-aghaj	21.86	**10**	14.0	**2**	4.9	**11**	10.5	**9**
Hokm-abad	21.06	**15**	23.3	**8**	0.5	**2**	13.3	**12**
Sitel	23.60	**1**	23.4	**9**	3.0	**8**	6.2	**2**
Verko	21.83	**11**	30.1	**16**	4.6	**10**	13.0	**11**
Vlasta	23.35	**3**	7.9	**1**	13.1	**18**	5.9	**1**
Monz 42	20.99	**16**	23.7	**10**	2.5	**7**	13.6	**13**
Fix 232	23.22	**4**	33.4	**18**	9.0	**15**	10.1	**8**
NS_Banat	23.50	**2**	27.2	**11**	3.3	**9**	6.5	**3**
Sanditi	21.30	**13**	14.5	**3**	6.8	**14**	13.8	**14**
Alpha	20.26	**17**	28.6	**14**	2.2	**6**	20.3	**17**
Plato ZS	22.10	**8**	17.6	**4**	2.0	**5**	8.2	**5**
Niva	21.88	**9**	28.5	**13**	11.0	**17**	15.5	**15**
Mean	22.0		23.7		4.8		12.0	

(c) Genotype	BNF (kg ha^{-1}) Mean	Rank	Stability parameters: CV_i Value	Rank	$б_i^2$ Value	Rank	P_i Value	Rank
Mohajeran	378.3	**6**	26.8	**12**	2733.6	**11**	3333.1	**5**
Khorvande	292.8	**18**	26.4	**11**	846.1	**3**	12727.4	**18**
Famenin	311.7	**17**	16.2	**5**	857.4	**4**	9354.2	**15**
Ghargholögh	348.7	**10**	16.9	**6**	56.2	**1**	5184.8	**8**
Ordobad	324.8	**14**	35.5	**18**	2741.4	**12**	9534.1	**16**
Shorakat	368.8	**7**	19.4	**7**	1324.5	**7**	4115.8	**7**
Ghara-aghaj	337.0	**12**	11.3	**3**	3962.6	**15**	7483.2	**13**
Hokm-abad	328.4	**13**	27.2	**13**	1810.6	**9**	7435.4	**12**
Sitel	389.1	**5**	20.7	**8**	314.4	**2**	2505.5	**4**
Verko	366.7	**9**	34.9	**17**	4552.8	**17**	5916.4	**10**
Vlasta	395.2	**3**	5.0	**2**	3837.9	**14**	2246.7	**3**
Monz 42	319.9	**15**	23.1	**9**	1505.1	**8**	8242.4	**14**
Fix 232	398.5	**2**	34.0	**16**	4585.5	**18**	3741.9	**6**
NS_Banat	406.9	**1**	24.7	**10**	1105.3	**6**	1559.7	**1**
Sanditi	338.1	**11**	2.5	**1**	4534.9	**16**	7135.1	**11**
Alpha	318.0	**16**	29.0	**15**	2161.0	**10**	10254.4	**17**
Plato ZS	391.8	**4**	16.2	**4**	1022.1	**5**	1727.9	**2**
Niva	368.3	**8**	27.4	**14**	3829.3	**13**	5236.5	**9**
Mean	354.6		22.1		2321.1		5985.3	

CV_i= Coefficient of variability; $б_i^2$ = Stability variance; P_i= Superiority measure

In relation to total biomass yield (shoot, stubble and root), Vlasta, Ghara-aghaj, Sanditi and PlatoZS had the least variability (CV_i) and Fix232, Ordobad, Verko and Khorvande the highest (Table 8b). The trend of relationship between total biomass yield and CV_i was similar to shoot dry matter (Table 11). By considering total biomass and CV_i values, Vlasta, Sitel, Shorakat, Ghargholögh and PlatoZS can be regarded as high yielding and relatively stable genotypes. Sanditi, Vlasta, PlatoZS and Ghara-aghaj had the lowest CV_i for BNF (Table 8c).
The trend of relationship of BNF and CVi was similar to shoot dry matter and total biomass (Table 11). Vlasta, PlatoZS, Sitel and Shorakat can be selected by simultaneously considering of BNF and CV_i.
Stability variance ($б_i^2$) has been proposed by Shukla (1972a). It is classified in type 2 stability parameters by Lin *et al*. (1986). Type 2 stability is a relative measure depending on the other genotypes in the test as there is no guaranty for a stable genotype according to this definition to appear stable if tested against another set of genotypes (Lin *et al*., 1986). The $б_i^2$ divides genotype by environment interaction (GEI) and assigns it to individual genotypes. So, a genotype with small $б_i^2$ is regarded as stable. As stability variance is the difference between two sums of squares, it can be negative. This result may be taken as equal to zero (Becker and Leon 1988). Shukla's $б_i^2$ indicates how close the performance of a genotype of interest

parallels the mean performance of all genotypes evaluated, providing an unbiased estimate of genotype x environment interaction (Robins *et al*., 2004). Hokmabad, Sitel, PlatoZS, Monz42 and Fix232 had small $б_i^2$ for shoot dry matter and are considered as stable (Table 8a). Correlations between shoot dry matter and $б_i^2$ were small and insignificant (Table 11). Sitel, PlatoZS, Fix232 and Vlasta were selected by simultaneously considering shoot dry matter yield and $б_i^2$. Ghargholog, Hokmabad, Famenin, Khorvande and PlatoZS had the lowest $б_i^2$ with regard to total biomass yield (Table 8b). Based on simultaneously regarding total biomass yield and $б_i^2$, the genotypes of Ghargholog, Sitel, NS-Banat and PlatoZS have been selected. A positive correlation was found between total biomass yield and $б_i^2$ under irrigated (r = 0.15) and rain-fed conditions (r = 0.47*) as well as for the mean of the two conditions (r = 0.44), indicating that high yielding genotypes showed a larger genotype by environment interaction (Table 11).

In relation to BNF estimates, Ghargholog had the lowest $б_i^2$ followed by Sitel, Khorvande, Famenin, PlatoZS and NS-Banat (Table 8c). Based on considering BNF estimates and $б_i^2$, Sitel, NS-Banat, PlatoZS, Shorakat have been selected with high and stable BNF. Correlation between BNF and $б_i^2$ was positive and insignificant (Table 11). In relation to BNF estimates, Ghargholog had the lowest $б_i^2$ followed by Sitel, Khorvande, Famenin, PlatoZS and NS-Banat (Table 8c). Based on considering BNF estimates and $б_i^2$, Sitel, NS-Banat, PlatoZS, Shorakat have been selected with high and stable BNF. Correlation between BNF and $б_i^2$ was positive and insignificant (Table 11).

Another stability statistic which was studied in this research is the superiority measure (P_i) of Lin and Binns (1988). Lin and Binn's P_i describes the similarity between the performance of a genotype of interest and the best genotype in each environment; stable genotypes will have a performance close to the maximum in each environment. The incorporation of the magnitude of the phenotypic performance into the stability formula may be more useful in an applied breeding situation. A small value of this parameter is considered as stable. Sitel followed by PlatoZS, Fix232, Vlasta, Mohajeran and Verko in relation to shoot DM (Table 8a), Vlasta followed by Sitel, NS-Banat, Ghargholog, PlatoZS and Mohajeran in relation to total biomass yield (Table 8b), and NS-Banat, followed by PlatoZS, Vlasta, Sitel, Mohajeran and Fix232 in relation to BNF estimates (Table 8c) had the lowest P_i, respectively. Howbeit, Sitel, PlatoZS, Vlasta, Fix232, Mohajeran and Verko based on shoot DM; Sitel, Vlasta, NS-Banat, Mohajeran, Shorakat and Fix232 on basis of total biomass yield; and NS-Banat, PlatoZS, Vlasta, Fix232, Sitel and Mohajeran based on BNF estimates can be selected by simultaneously considering genotype performance and stability (P_i). Correlation between P_i

and crop performances (shoot DM, total biomass yield and BNF) in all cases (rain-fed, irrigated and average over two conditions) was negative and significant, indicating high yielding genotypes had small P_i or selection based on low P_i may be resulted in high yield genotypes (Table 11). Robins *et al.* (2004) reported that alfalfa forage yield was positively correlated with σ_i^2 and negatively correlated with Pi.

GEI analysis and multivariate stability statistic (AMMI analysis)

The AMMI analysis is a powerful multivariate method to quantify GEI interaction in multi-environmental trials. In this analysis, GE interaction is partitioned by principal component analysis (Gauch 1988, Zobel *et al.*, 1988, Crossa *et al.*, 1991). The AMMI analysis for shoot dry matter indicated that the environment, genotype and GE interaction explained 79.5, 11.5 and 9.0 % of the total variation (Table 9).

The first two interaction principal component axes, IPCA1 and IPCA2, explained 5.8 and 2.2 % of the total variation, which is 64.5 and 24 % of the GE interaction. Only the mean square of IPCA1 was significant ($P < 1\%$). In relation to total biomass yield, the environment, genotype and GE interaction captured 79.6, 5.6 and 14.8 % of the total variation (Table 8).

The first two interaction principal component axes, IPCA1 and IPCA2, explained 8.7 and 4.9 % of total variation, which is 59.0 and 32.8% of the GE interaction. The mean square of IPCA1 and IPCA2 were significant ($P < 5\%$).

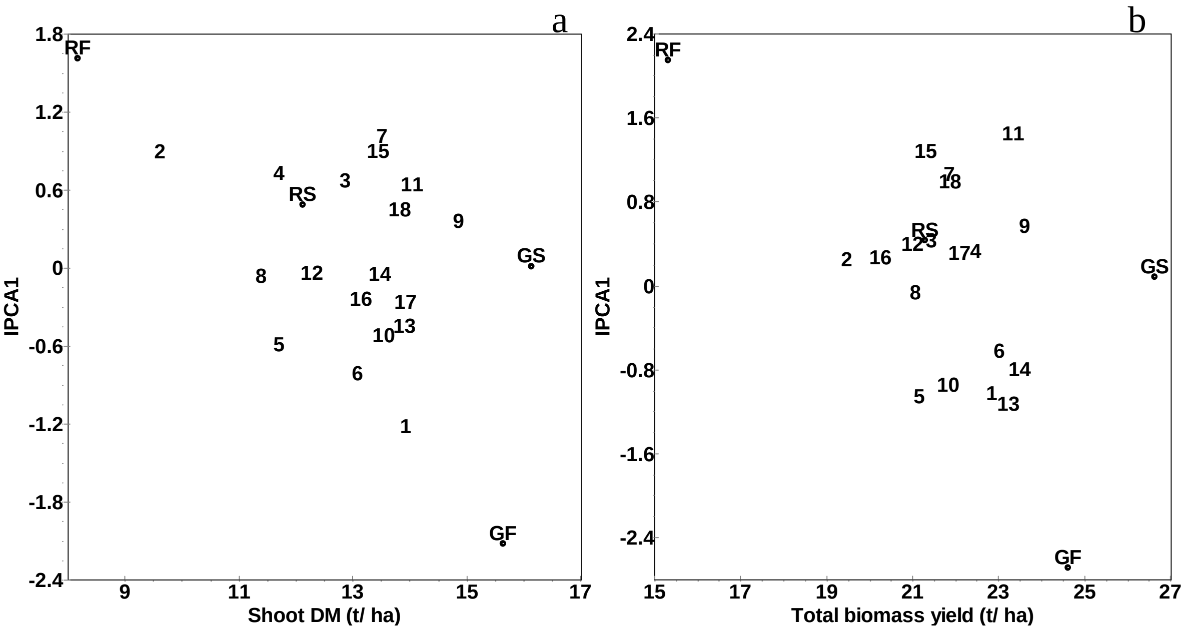
Table 9. AMMI analysis of shoot DM, total biomass yield and BNF in alfalfa genotypes.

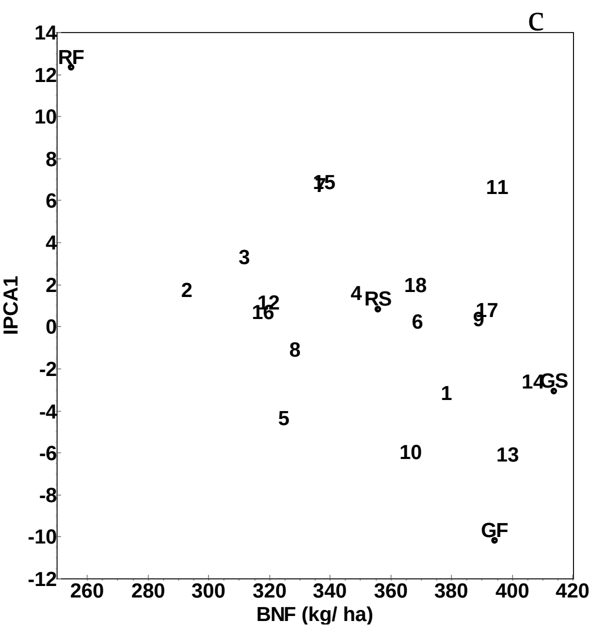
		Shoot DM			Total biomass yield			BNF		
Source	df	SS .	TSS (%)	GESS (%)	SS	TSS (%)	GESS (%)	SS	TSS (%)	GESS (%)
Total	71	926.9	100	-	1661.8	100	-	471490	100	-
Genotype	17	106.7	11.5	-	92.2	5.6	-	81529.9	17.3	-
Environment	3	736.5	79.5	-	1323.3	79.6	-	271582	57.6	-
GEI	51	83.7	9.0	-	246.3	14.8	-	118379	25.1	-
IPCA1	19	54.0**	5.8	64.5	145.2*	8.7	59.0	70445.3*	14.9	59.5
IPCA2	17	20.1	2.2	24.0	80.7*	4.9	32.8	31820.1	6.7	26.9
Residual	15	9.6	1.0	11.5	20.3	1.2	8.2	16113.2	3.4	13.6

TSS(%)= Contribution of total sum of square; GESS(%)=Contribution of GEI sum of square;* and **= Significant at 5 and 1% probability level.

With regard to biological nitrogen fixation (BNF), the environment, genotype and GE interaction justified 57.6, 17.3 and 25.1 % of the total variation (Table 9). The first two interaction principal component axes, IPCA1 and IPCA2, explained 14.9 and 6.7 % of total variation, which are 59.5 and 26.9 % of the GE interaction. Only the mean square of IPCA1 was significant (P < 5 %). The large contribution of environment in total variation of these characters showed that environments were diverse, with large differences. The larger contribution of GE interaction rather than genotype for total biomass yield and BNF exhibited that there were substantial differences in the genotypic response across environments.

The biplot of main effects against IPCA1 scores of genotypes and environments (AMMI1 biplot) explained 96.8, 93.9 and 89.8 % of total variation of shoot DM, total biomass yield and BNF, respectively (Figs. 4a,b and c).





RF= First year of rain-fed trial; RS= Second year of rain-fed trial; GF= First year of irrigated trial; GS= Second year of irrigated trial; Numbers 1 to 18 are genotypes Mohajeran, Khorvande, Famenin, Gharghologh, Ordobad, Shorakat, Ghara-aghaj, Hokmabad, Sitel, Verko, Vlasta, Monz42, Fix232, NS-Banat , Sanditi, Alpha, PlatoZS and Niva, respectively.

Fig 4. AMMI1 biplot for genotypes (numbers) and environments (letters) using shoot DM (a), total biomass yield (b) and BNF (c) data.

The IPCA scores of a genotype in the AMMI analysis are an indicator of the stability of a genotype over environments. The greater the IPCA scores, either negative or positive, the more specifically adapted genotypes are to certain environments. IPCA scores approaching zero (0) indicate that the genotype is more stable over all tested environments. With regard to all 3 traits, shoot DM, total biomass yield and BNF, RF (Rain-fed-First year) had a large negative IPCA1 score while GF (Irrigated-First year) had a large positive one (Fig. 4a, b and c). These two environments interact strongly with the genotypes but in the opposite direction, positively with genotypes that have same sign of IPCA1 scores and vice versa. RS (Rain-fed, Second year) and GS (Irrigated, Second year) had small IPCA1 scores, indicating that they had little interaction with the genotypes. The environment can be sub-grouped according to their mean performance over the genotypes. GF and GS (Irrigated condition) had the higher average performance than RF and RS (Rain-fed condition) for the 3 studied traits.

Based on the average shoot DM of genotypes and similarity of their IPCA1 scores with environments, the genotypes Sitel(9), Vlasta (11), Niva (18), Sanditi (15) and Ghara-aghaj (7) are suitable for RS and RF (rain-fed condition), while Mohajeran (1) , Fix232 (13), PlatoZS (17)and Verko (10) are suitable for GF (Fig 4a). The environment GS with small IPCA1 score (near to zero) had negligible interaction with genotypes, suggesting that genotype with just high shoot DM yield are suitable. The genotypes 14 (NS-Banat), 17 (PlatoZS) and 9 (Sitel) had small IPCA1 scores and average shoot DM yields more than the grand mean, so they can be selected as high and stable yielding genotypes.

With regard to total biomass yield, the genotypes 9 (Sitel), Vlasta (11) and Gharghologh (4) were suitable for RS and RF (rain-fed condition), while the genotypes 14 (NS-Banat), 13 (Fix232), 6 (shorakat) and 1 (Mohajeran) were suitable for GF (Fig. 4b). The situation of GS was similar to that of shoot DM yield. The genotypes 9 (Sitel) and 4 (Gharghologh) with small IPCA1 scores and average total biomass yields more than grand mean can be considered as the best genotypes.

In relation to BNF, the genotypes 13, 14, 1 and 10 (Fix232, NS-Banat, Mohajeran and Verko, respectively) were suitable for GF and GS, (irrigation condition), while the genotype 11, 17 and 18 (Vlasta, PlatoZS and Niva, respectively) were suitable for RS and RF (rain-fed condition) (Fig. 4c). The genotypes 9 (Sitel) and 6 (Shorakat) which had small IPCA1 scores and average BNF higher than grand mean can be considered as stable genotypes with broad adaptability to tested conditions.

Selection of stable genotypes based on IPCA1 or IPCA2 scores yields different results because of their different values. Therefore, calculation of a balanced measure of the two different scores seems to be the better option. Purchase (1997) suggested AMMI stability value (ASV) which is calculated using a principle of the Pythagoras theorem. The ASV is the distance from the coordinate point to the origin in a biplot of IPCA1 scores against IPCA2 scores in AMMI analysis (Figs. 5a, b and c). The genotypes that are located further away from the origin are more responsive (Yan and Hunt 1998). So, the small value of ASV is considered as stable. The scores of the first and second interaction principle component axes (IPCA1 and IPCA2) from AMMI analysis and calculated AMMI stability values (ASV) of tested genotypes and its rank are shown with regard to shoot dry matter, total biomass yield and BNF estimate in Table 10. First two IPCA explained cumulatively 88.5, 91.8 and 86.4 of GE interaction for shoot DM, total biomass and BNF, respectively.

Table 10. The scores of IPCA1, IPCA2 and ASV value of genotypes for shoot DM (t ha^{-1}), total biomass yield (t ha^{-1}) and BNF (kg ha^{-1}) resulting from AMMI analysis.

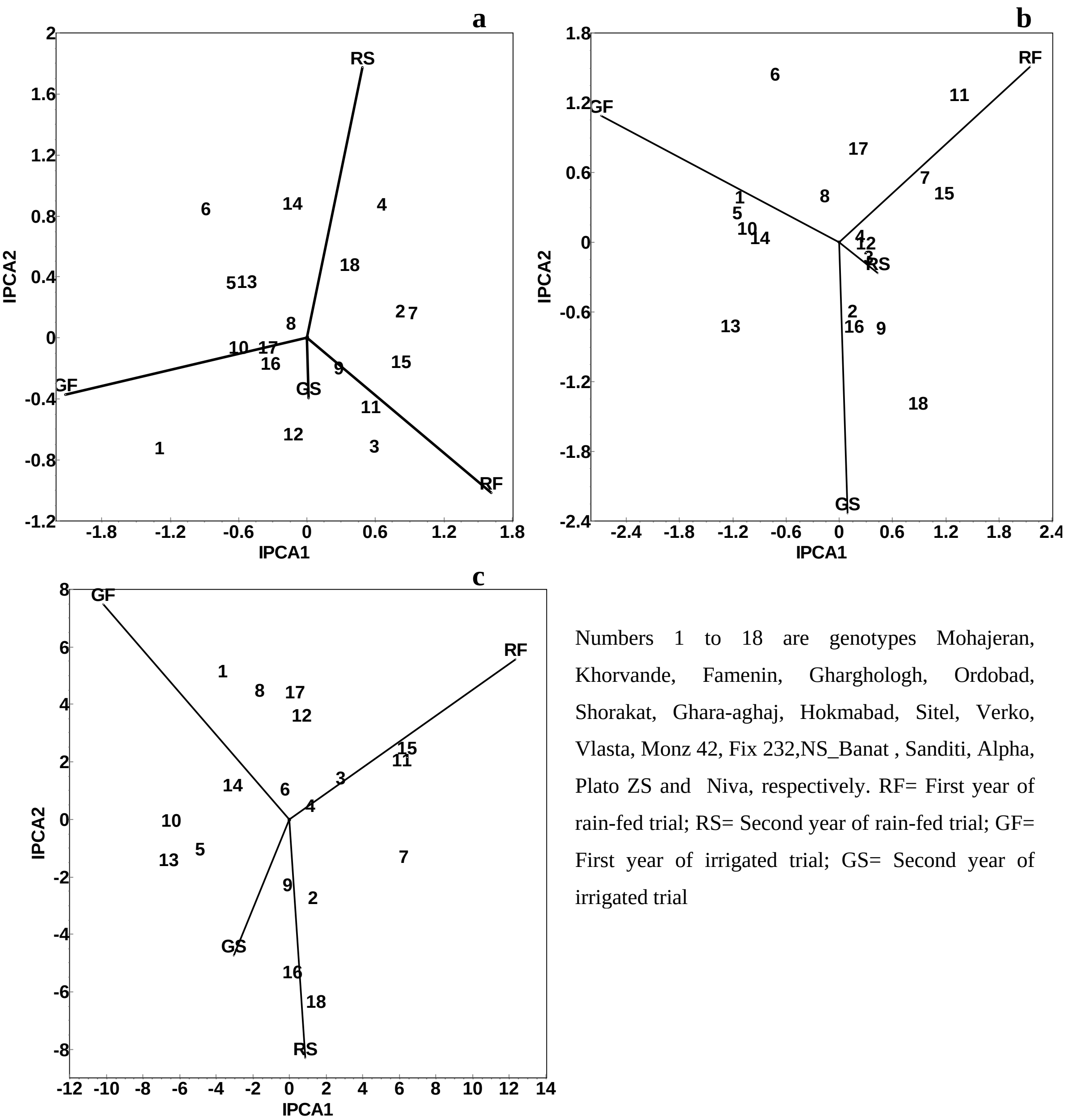
	Shoot dry matter (t ha^{-1})				Total biomass yield (t ha^{-1})				BNF (kg ha^{-1})			
Genotype	IPCA1	IPCA2	ASV	Rank	IPCA1	IPCA2	ASV	Rank	IPCA1	IPCA2	ASV	Rank
Mohajeran	-1.294	-0.783	3.57	18	-1.119	0.307	2.04	13	-3.643	4.825	9.40	12
Khorvande	0.816	0.115	2.20	14	0.149	-0.676	0.73	5	1.269	-3.049	4.15	6
Famenin	0.591	-0.769	1.76	11	0.336	-0.207	0.64	4	2.792	1.095	6.28	9
Ghargholog h	0.654	0.815	1.94	13	0.240	-0.026	0.43	1	1.135	0.157	2.52	2
Ordobad	-0.669	0.304	1.82	12	-1.148	0.174	2.07	14	-4.865	-1.369	10.86	13
Shorakat	-0.889	0.786	2.52	17	-0.718	1.361	1.88	12	-0.257	0.703	0.90	1
Ghara-aghaj	0.930	0.105	2.50	16	0.970	0.476	1.81	10	6.238	-1.619	13.91	15
Hokmabad	-0.144	0.035	0.39	1	-0.163	0.320	0.43	2	-1.602	4.175	5.48	7
Sitel	0.279	-0.256	0.79	3	0.476	-0.823	1.19	8	-0.123	-2.607	2.62	3
Verko	-0.600	-0.123	1.62	10	-1.037	0.034	1.87	11	-6.451	-0.360	14.29	16
Vlasta	0.564	-0.510	1.60	9	1.352	1.188	2.71	18	6.160	1.724	13.75	14
Monz 42	-0.116	-0.694	0.76	2	0.299	-0.091	0.55	3	0.677	3.282	3.61	4
Fix 232	-0.528	0.306	1.45	8	-1.225	-0.799	2.34	17	-6.577	-1.758	14.67	18
NS-Banat	-0.128	0.819	0.89	4	-0.890	-0.042	1.60	9	-3.106	0.858	6.93	10
Sanditi	0.826	-0.215	2.23	15	1.189	0.344	2.17	15	6.402	2.157	14.34	17
Alpha	-0.320	-0.229	0.89	5	0.175	-0.804	0.86	7	0.180	-5.642	5.66	8
Plato ZS	-0.344	-0.123	0.93	6	0.220	0.729	0.83	6	0.294	4.093	4.14	5
Niva	0.373	0.418	1.09	7	0.894	-1.464	2.17	16	1.479	-6.665	7.43	11

IPCA1 and IPCA2 are first and second interaction principal component axes; ASV is AMMI stability value.

Based on ASV values, Hokmabad, Monz42, Sitel, NS-Banat, Alpha and PlatoZS in relation to shoot dry matter; Ghargholog h, Hokmabad, Monz42, Famenin, Khorvande and PlatoZS concerning total biomass yield and Shorakat, Ghargholog h, Sitel, Monz42, PlatoZS and Khorvande in relation to BNF were considered as stable genotypes, respectively (Table 10 ; Figs. 5a, b and c).

The fact that one high yielding cultivar (Sitel) and one low yielding cultivar (Khorvande) were the two most stable cultivars serves to demonstrate the importance of analyzing both stability and yield performance to determine also the adaptability patterns for cultivar recommendations. With regard to genotype performance and ASV value, Sitel, PlatoZS, Vlasta, NS-Banat, Fix232 and Niva have been selected as high yielding and stable genotypes with regard to shoot dry matter. Ghargologh, Sitel, NS-Banat and PlatoZS regarding total biomass yield and Sitel, Shorakat, PlatoZS and NS-Banat concerning BNF can be considered as stable and high yield genotypes.

The AMMI biplot of IPCA1 vs. IPCA2 (AMMI2) (Figs. 5a, b and c) showed that RF (First year of rain-fed trial) followed by GF (First year of irrigated trial) had the greatest contributions to GE interaction, in other words, the first year of study had more effect on GE



Numbers 1 to 18 are genotypes Mohajeran, Khorvande, Famenin, Ghargologh, Ordobad, Shorakat, Ghara-aghaj, Hokmabad, Sitel, Verko, Vlasta, Monz 42, Fix 232,NS_Banat , Sanditi, Alpha, Plato ZS and Niva, respectively. RF= First year of rain-fed trial; RS= Second year of rain-fed trial; GF= First year of irrigated trial; GS= Second year of irrigated trial

Fig. 5. AMMI biplot of IPCA1 vs. IPCA2 using data of shoot DM (a), total biomass yield (b) and BNF (c).

interaction. Genotypes toward the center of biplot have zero interaction; therefore have general adaptation with different performance, while genotype further from the center of the biplot show specific adaptation. Using shoot DM data, Sitel (9), Hokmabad (8), Alpha (16) and PlatoZS (17) can be considered as stable and wide adapted genotypes. Ghargologh, Monz42, Famenin and Hokmabad were stable using total biomass yield and with regard to BNF, Ghargologh, Shorakat were stable. The result of AMMI2 biplot was in agreement with the AMMI stability value (ASV) to select stable genotypes.

Gomez-Beccera et al. (2007) reported small and negative correlation (r = -0.12) between grain yield of spring wheat varieties and ASV. Except for total biomass mean over locations, non-significant correlations have been found between ASV and shoot dry matter, total biomass yield and BNF under irrigated, rain-fed conditions as well as mean over locations (Table 11).

Correlation between stability statistics (Table 11) showed that only ASV and $б_i^2$ had significant and positive correlation. The relationship between parameters also showed that direction and amount of correlation can be changed based on trait under consideration.

Table 11. Simple correlation between stability statistics and shoot DM, total biomass yield and BNF estimates under irrigated, rain-fed and average over two conditions.

	Trait	Rain-fed	Mean	CVi	$б_i^2$	Pi	ASV
Irrigated	SHDM	0.49*	0.90**	0.22	0.14	-0.93**	0.00
	TBY	-0.04	0.71**	0.51*	0.15	-0.54*	0.28
	BNF	0.22	0.84**	0.43	0.09	-0.77**	0.11
Rain-fed	SHDM		0.83**	-0.68**	-0.09	-0.72**	-0.08
	TBY		0.67**	-0.79**	0.47*	-0.73**	0.37
	BNF		0.72**	-0.64**	0.19	-0.75**	0.19
Mean	SHDM			-0.20	0.04	-0.96**	-0.04
	TBY			-0.19	0.44	-0.92**	0.47*
	BNF			-0.05	0.17	-0.96**	0.18
CVi	SHDM				0.24	0.06	0.10
	TBY				-0.16	0.43	-0.07
	BNF				0.01	0.20	-0.06
$б_i^2$	SHDM					0.01	0.93**
	TBY					-0.22	0.90**
	BNF					-0.04	0.90**
Pi	SHDM						0.12
	TBY						-0.25
	BNF						-0.04

Irrigated, rain-fed and mean refer to irrigated and rain-fed conditions as well mean over these two conditions; SHDM= Shoot dry matter; TBY= Total biomass yield; BNF= Biological nitrogen fixation; CV_i= Coefficient of variability; $б_i^2$ =Stability variance; P_i= Superiority measure; ASV= AMMI stability value.

For example, correlation between CVi and σ_i^2 was positive (r=0.24) using shoot DM data, while it was negative (r=-0.16) for total biomass yield and negligible and near to zero (r= 0.01) for BNF.Robins *et al.* (2004) found negative correlation between P_i and σ_i^2 between individual genotypes within cultivars and also overall of genotypes. Fikere *et al.* (2008) found significant positive rank correlation between ASV and σ_i^2 (r = 0.818**); small and positive between ASV and CVi (r = 0.155); moderately positive between CVi and σ_i^2 (r = 0.369) in grain yield of 16 faba bean *(Vacia faba L.*) genotypes tested in South Eastern Ethiopia.

Phenotypic correlations and path analysis

Phenotypic (simple) correlations between traits under irrigated and rain-fed conditions have been separately shown in Table 12. Correlations between crop regrowth (cm) and plant height (cm), LAI ,Shoot DM (t ha^{-1}) , root protein content (%) and BNF estimate were significant and positive under rain-fed condition, while these were negative under irrigated condition.

Plant height was positively and significantly correlated with LAI and shoot DM under both conditions.The morphological trait of plant height is an important yield component and it is often used as a criterion when choosing superior genotypes in an early stage of selection. Research conducted by numerous authors confirms that there is a positive correlation between yield and plant height (e.g. Hauptvogel 1994, Katepa-Mupondwa *et al*., 2002).

Table 12. Phenotypic correlations between traits among tested genotypes (n=18) under irrigated and rain-fed conditions.

Trait	CR	PH	STN	NN	CHL	LAI	LSR	SH-DM	ST-DM	RO-DM	TBS	BNF	SCP	RCP
CR		.81**	0.30	0.10	0.03	.54*	-0.35	.60**	-0.26	0.32	.65**	.60**	-0.19	-0.30
PH	-0.20		0.26	0.23	0.13	.72**	-0.07	.84**	0.01	0.10	.72**	.72**	-0.17	-0.43
STN	0.02	0.36		-0.10	-0.01	0.10	-0.34	0.26	-0.21	0.41	0.45	0.30	-0.34	-0.40
NN	-0.16	-0.08	-0.34		-0.09	0.42	0.37	0.11	0.20	-0.20	-0.03	0.06	0.17	-0.04
CHL	-0.18	0.42	0.41	-0.18		0.08	0.16	0.31	0.15	0.01	0.26	0.26	0.01	-0.22
LAI	-.60**	.53*	-0.12	0.22	-0.08		0.04	.63**	0.41	0.03	.55*	.49*	-0.18	-.63**
LSR	-0.43	-0.12	-.71**	0.23	-0.26	.57*		-0.04	0.15	-.51*	-0.33	-0.09	.55*	-0.09
SHDM	-0.29	.60**	0.14	0.20	0.03	.72**	0.21		0.20	0.01	.81**	.87**	-0.16	-.58*
STDM	0.30	-0.15	-0.33	0.23	-0.26	0.02	0.22	0.16		-0.27	0.08	0.10	-0.05	-0.32
RODM	.47*	-0.44	-0.09	-0.26	-0.16	-0.40	-0.10	-0.44	0.23		.59*	0.38	-0.22	-0.32
TBS	0.16	0.15	0.03	0.00	-0.14	0.33	0.09	.56*	0.45	.49*		.93**	-0.27	-.69**
BNF	-0.04	0.08	-0.11	0.24	-0.15	0.43	0.29	.61**	.55*	0.32	.91**		0.01	-.68**
SCP	-.55*	-0.18	-0.30	.48*	0.09	0.32	.58*	0.06	0.21	-0.26	-0.15	0.20		-0.07
RCP	0.38	-0.44	0.21	-0.25	0.11	-.77**	-.53*	-.67**	-0.26	0.21	-0.47	-.51*	-0.20	

* and **. Correlation is significant at the 0.05 and 0.01 level, respectively (2-tailed).

Above diagonal correlations under rain-fed and below diagonal under irrigated conditions.

Plant height showed weak positive correlations with total biomass yield (r = 0.15) and BNF (r = 0.08) under irrigated condition, while it was strong and significant under rain-fed condition (r = 0.72**) similar for both traits. Positive and non-significant correlations were determined between stem numbers per m^2 and shoots DM under both conditions. It can be interpreted in the way that in low input and stockless cropping system in organic farming increasing the stem number per m^2 caused more competition among stems to get water and nutrients. This is more obvious in the irrigated trial with increased stem number per m^2 compared to the rain-fed condition (Tables 7 and 12). Negative correlation under irrigated (r = -0.44) and negligible correlation (r = 0.01) under rain-fed conditions were determined between shoot DM and root DM. The genotypes Mohajeran, Sitel and Verko had the greatest shoot DM over two years under irrigated condition, but their root DM were less than average of all genotypes over years (Table 7).

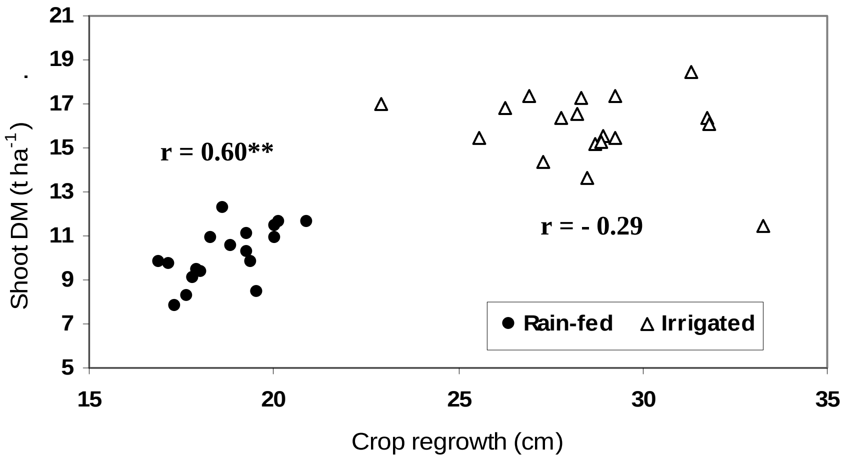


Fig. 6. The relationship of shoot DM and crop regrowth under two different conditions.

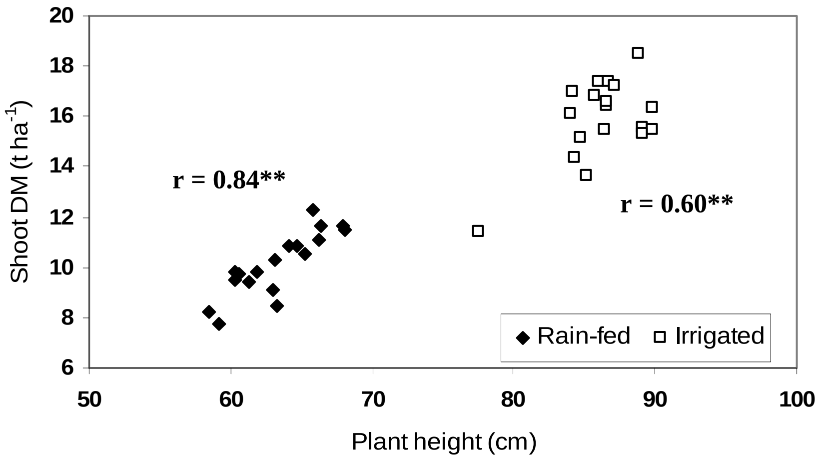


Fig. 7. The ralationship of shoot DM and plant height under two different conditions.

Under both conditions, root DM represented negative correlation with shoot protein content and leaf to stem ratio (Table 12). Total biomass yield under irrigated condition was significantly correlated with shoot DM (r = 0.56*) and root DM (r = 0.49*), while under rain-fed condition, was significantly correlated with crop regrowth (r = 0.65**), Plant height (r = 0.72**), LAI (r = 0.55*), shoot DM (r = 0.81**) and root DM (r = 0.59*). It seems that total biomass yield under stress condition was affected by more characters than under non-stressed condition. A moderately positive correlation was found between total biomass yield and BNF estimates under both conditions (Table 12).

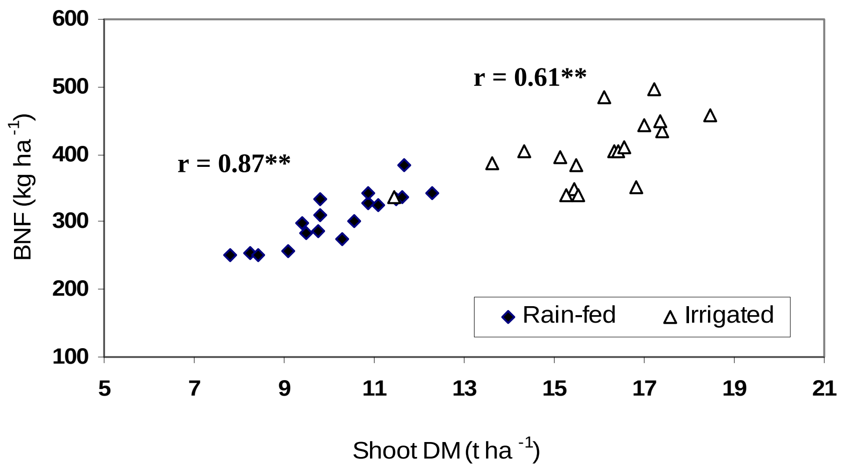


Fig.8. The relationship of BNF and shoot DM under two different conditions.

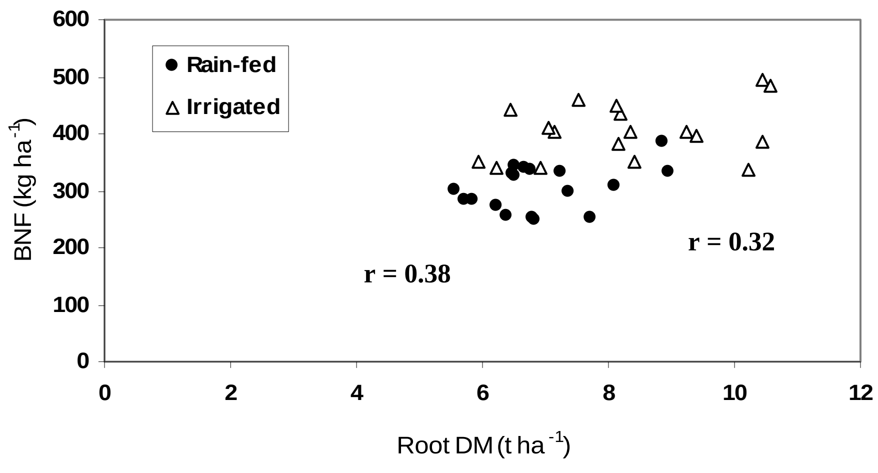


Fig. 9 The relationship of BNF and root DM under two tested conditions.

BNF under rain-fed condition was higher correlated with crop regrowth (r = 0.6**), plant height (r = 0.72**), stem number per m^2 (r = 0.30) and LAI (r = 0.49*) than that of irrigated condition (r = -0.04, 0.08, -0.11 and 0.43, respectively). Positive correlation has been found between BNF and shoot DM, root DM, stubble DM and total biomass yield in both conditions (Table 12). Significant negative correlation has been determined between BNF and root protein content in both trials, while it had negligible positive correlation with shoot protein (Table 12). The negative correlation of shoot protein content with plant height and stem number per m^2 and positive correlation with leaf to stem ratio in both environments (Table 12) showed the impact of leaf content of harvested biomass on protein content and forage quality. Rosellini and Veronesi (2006) found that alfalfa protein content was positively correlated with leaf-to-stem ratio. Tucak *et al*. (2008) reported negative correlation between plant height with leaf ratio (r = -0.68**) and protein content (r = -0.11), and a negligible correlation between leaf ratio and protein content (r = 0.03). Riday and Brummer (2005) found significant and negative correlation in alfalfa between plant height and shoot protein content (r = -0.27*) and no significant correlation with leaf to stem ratio. They found no significant correlation between crude protein and leaf to stem ratio.

Negative correlation was found between shoot protein content and crop regrowth under both conditions, while it was positive between root protein content and crop regrowth under irrigated condition. Riday and Brummer (2005) found significant and negative correlation between spring regrowth and shoot crude protein (r = -0.30*) and highly significance positive correlation between leaf to stem ratio and midseason (r = 0.39***) and autumn (r = 0.42***) regrowth.

Path analysis

The results of Path analysis and decomposition of correlation coefficients with morphological characters into direct and indirect effects are separately shown for BNF and its components, shoot DM and root DM for two different conditions in Tables 13 to 15.

All components of BNF had positive direct effects in both conditions (Table 13). Shoot DM, root DM and shoot protein content (or nitrogen percent) had the greatest direct effects on BNF, respectively, in both conditions, indicating that these characters can be considered as main selection criteria for BNF improvement in both conditions. The low residual effects indicate that most of the BNF variations were accounted for by the traits in model in both conditions (Fig. 10).

Direct and indirect effects of plant height, stem number per m^2, LAI, crop regrowth and leaf to stem ratio on shoot and root DM was shown in Tables 14 and 15. Under irrigated condition, LAI had the greatest direct effect on shoot DM followed by crop regrowth, plant height and stem number per m^2. Although plant height and crop regrowth had similar small and positive direct effects on shoot DM in the irrigated trial, different large indirect effect of these characters via LAI caused different phenotypic correlations with shoot DM (Table 14).

Table. 13. Direct (diagonal and bold values) and indirect effects of different components on BNF under irrigated and rain-fed conditions.

Casual Variable	Trial	SHDM	STDM	RODM	SHCP	STCP	ROCP	Correlation with BNF
SHDM	IR	**1.03*****	0.02	-0.36	0.02	-0.01	-0.10	0.61
	RF	**0.92*****	0.03	0.00	-0.05	0.03	-0.07	0.87
STDM	IR	0.17	**0.13**	0.18	0.07	0.03	-0.04	0.55
	RF	0.18	**0.14**	-0.14	-0.01	-0.02	-0.04	0.10
RODM	IR	-0.46	0.03	**0.80*****	-0.09	0.01	0.03	0.32
	RF	0.01	-0.04	**0.53*****	-0.07	-0.01	-0.04	0.38
SHCP	IR	0.07	0.03	-0.21	**0.33****	0.01	-0.03	0.20
	RF	-0.15	-0.01	-0.12	**0.31****	-0.02	-0.01	0.00
STCP	IR	-0.16	0.05	0.05	0.06	**0.08**	-0.05	0.03
	RF	0.31	-0.03	-0.04	-0.07	**0.10**	-0.03	0.24
ROCP	IR	-0.69	-0.03	0.17	-0.07	-0.03	**0.14**	-0.51
	RF	-0.54	-0.04	-0.17	-0.02	-0.02	**0.12**	-0.67

IR= Irrigation (R^2 = 0.96; Residual=0.21); RN=Rain-fed (R^2 = 0.97 ; Residual=0.19)
SHDM, STDM and RODM = Shoot, stubble and root dry matter, respectively; SHCP, STCP and ROCP = Shoot, stubble and root protein content, respectively.

In the rain-fed trial, plant height had considerably larger positive direct effect on shoot DM followed by negative direct effect of crop regrowth. The positive and significant correlation between shoot DM and crop regrowth resulted from large indirect effect via plant height on shoot DM. Although direct effect of stem number per m^2 was smaller under rain-fed than of that under irrigated condition, proper positive indirect effect via plant height under rain-fed condition resulted in higher correlation between shoot DM and stem number per m^2 under rain-fed than irrigated conditionLarge positive indirect effect of leaf to stem ratio via LAI under irrigated trial, while direct effect was negative and negligible, resulted in positive correlation with shoot DM. Regression and path analysis revealed that improved forage yield in P- and K-fertilized plots was consistently associated with greater mass per shoot (Berg *et al.*, 2007).

Crop regrowth had the largest positive direct effect on root DM in both conditions, whereas direct effect of plant height was negative and large (Table 15). The negligible positive correlation between plant height and root DM in rain-fed trial resulted from large positive indirect effect of plant height via crop regrowth on root DM. Stem number per m^2 had a positive direct effect on root DM in both conditions, because of negative indirect effect via plant height and leaf to stem ratio, showed negligible negative correlation with root DM under irrigated condition. The negative correlation between root DM and LAI resulted from negative indirect effects of LAI via plant height and crop regrowth on root DM under irrigated trial, whereas direct effect of LAI on root DM was similar negligible positive in both conditions (Table 15). Leaf to stem ratio had different direct effect on root DM in both conditions, small and positive under irrigated and large and negative under rain-fed condition. The large residual effect, particularly for root DM, indicates that most of the variation of dependent variables (shoot and root DM) were not accounted for by traits included in the model under both conditions (Tables 14 and15; Figures 10a and b). The result of path analysis was diagrammatically displayed for each condition in Figures 10a and b.

Table. 14. Direct (diagonal, bold values) and indirect effects of different traits on shoot DM under irrigated and rain-fed conditions.

Casual variable	Trial	PH	STN	LAI	CR	LSR	Correlation with shoot DM
PH	IR	**0.18**	0.05	0.41	-0.04	0.00	0.61
	RF	**1.02****	0.02	0.04	-0.24	0.00	0.85
STN	IR	0.07	**0.13**	-0.09	0.00	0.02	0.14
	RF	0.26	**0.06**	0.01	-0.09	0.02	0.26
LAI	IR	0.10	-0.02	**0.77***	-0.11	-0.02	0.72
	RF	0.74	0.01	**0.06**	-0.16	0.00	0.64
CR	IR	-0.04	0.00	-0.46	**0.19**	0.01	-0.29
	RF	0.83	0.02	0.03	**-0.30**	0.02	0.60
LSR	IR	-0.02	-0.10	0.44	-0.08	**-0.03**	0.21
	RF	-0.08	-0.02	0.00	0.10	**-0.05**	-0.04

IR= Irrigated (R^2 = 0.62; Residual=0.62); RN=Rain-fed (R^2 = 0.74; Residual=0.51); PH= Plant height; STN= Stem number per m^2; LAI= Leaf area index; CR= Crop regrowth; LSR= Leaf to stem ratio.

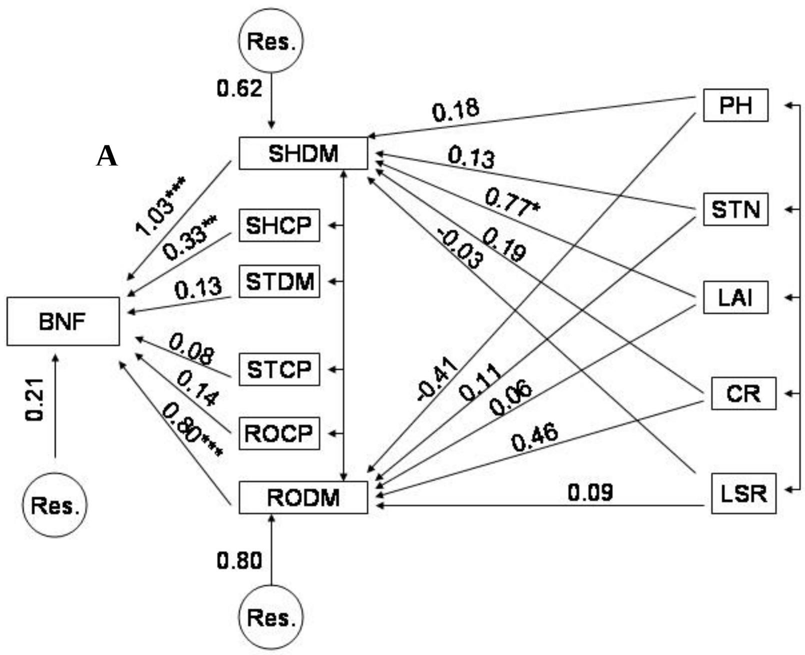
Table 15. Direct (diagonal, bold values) and indirect effects of different traits on root DM under irrigated and rain-fed conditions.

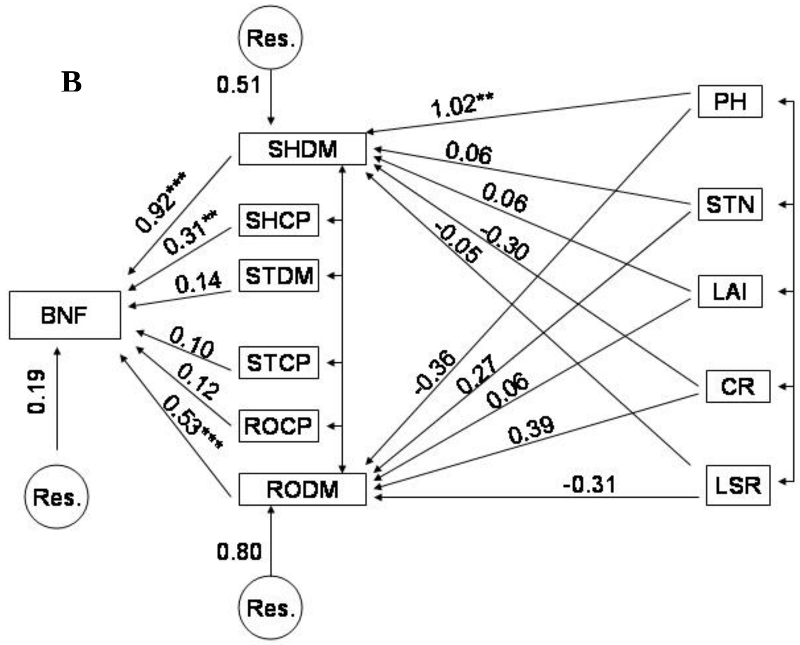
Casual variable	Trial	PH	STN	LAI	CR	LSR	Correlation with root DM
PH	IR	**-0.41**	0.04	0.03	-0.09	-0.01	-0.44
	RF	**-0.36**	0.07	0.04	0.32	0.02	0.09
STN	IR	-0.15	**0.11**	-0.01	0.01	-0.06	-0.09
	RF	-0.09	**0.27**	0.01	0.12	0.11	0.41
LAI	IR	-0.22	-0.01	**0.06**	-0.27	0.05	-0.40
	RF	-0.26	0.03	**0.06**	0.21	-0.01	0.03
CR	IR	0.08	0.00	-0.04	**0.46**	-0.04	0.47
	RF	-0.29	0.08	0.03	**0.39**	0.11	0.32
LSR	IR	0.05	-0.08	0.03	-0.20	**0.09**	-0.11
	RF	0.03	-0.09	0.00	-0.14	**-0.31**	-0.51

IR= Irrigated (R^2 = 0.36; Residual=0.62); RN=Rain-fed (R^2 = 0.36; Residual=0.62)

Finally, based on path analysis, a restricted simultaneous selection model is to be followed to nullify the undesirable indirect effects and antonymous direct effects of some traits, like plant height and crop regrowth, on shoot and root DM.

Fig. 10. Path diagram showing direct and indirect effect of casual traits on BNF, shoot DM and root DM in irrigated (A) and rain-fed (B) trials.





Single arrows are hypothesized causal relations between variables (path coefficient or direct effect) and double headed lines are correlations. BNF= Biological nitrogen fixation, SHDM, STDM and RODM= Shoot, stubble and root dry matter, SHCP, STCP and ROCP= Shoot, stubble and root protein content, PH= Plant height, STN= Stem number per m^2 , LAI= Leaf area index, CR= Crop regrowth, LSR= Leaf to stem ratio, RES:= Residual; *, **, *** = Significant at 0.05, 0.01 and 0.001 probability level.

Classification of genotypes and cluster analysis

Cluster analysis was performed to group and assess the suitability of the tested genotypes based on morphological traits in addition to shoot DM, root DM and shoot protein content in each condition (Figs. 11a, b and c). Also, cluster analysis can be applied to measure genetic distance and divergence between genotypes (Riday *et al*. 2003) which can be useful in planning the crossing program of a breeding project, particularly for organic breeding of alfalfa, in future. The aim of alfalfa breeding is releasing a cultivar with high forage yield and protein content (Riday and Brummer 2002). Under irrigated condition, 18 genotypes of alfalfa can be classified into 3 clusters (Fig. 11a and Table 17). First cluster included all Iranian ecotypes except for Khorvande (Mohajeran, Famenin, Gharghologh, Shorakat, Ordobad, Ghara-aghaj and Hokmabad), second cluster contained only one member, Khorvande, and third cluster contained 10 European cultivars (Sitel, Verko, Vlasta, Monz42, Fix 232, NS-Banat, Sanditi, Alpha, Plato ZS, and Niva) (Fig. 11a).

Apart from Khorvande, Iranian ecotypes in cluster one were characterized by fast crop regrowth after cutting, more stem number per m^2, much root biomass, less leaf and protein content in shoot biomass and less shoot dry matter compared to European cultivars in the third cluster (Table 17).Under rain-fed condition, genotypes can be grouped into four clusters (Fig. 11b and Table 17). First cluster included 3 Iranian ecotypes (Mohajeran, Khorvande and Ordobad) and 3 European cultivars (Verko, Monz42 and Alpha). This cluster is characterized by slow crop regrowth after cutting, short stem, low number of stem per m^2 and low LAI, shoot and root dry matter as well high leaf and protein content in shoot material (Table 17).

The second cluster consisted of 2 Iranian ecotypes (Famenin and Ghara-aghaj) and 4 European cultivars (Sitel, Vlasta, Sanditi and NS-Banat), which can be described by rapid crop regrowth, tall stem, acceptable stem number per m^2, high LAI , high shoot dry matter, root dry matter more than grand mean and low shoot protein content (Table 17).

The third cluster contained 3 Iranian ecotypes (Shorakat, Hokmabad and Gharghologh), which are characterized by high root dry matter and high stem number per m^2 as other characters were less or equal to the mean of all clusters (Table 17). The fourth cluster consisted of three European cultivars (Plato ZS, Niva and Fix 232), which are characterized by high shoot dry matter, high leaf and protein content of shoot material as well as tall stem and low root dry matter (Table 17).

With regard to results of cluster analysis in the rain-fed condition (Fig. 11b and Table 17) as well as mean comparisons (Table 7) and relationships between traits (Table 12) and path analysis (Tables 13 to 15 and Fig. 10), genotypes in second cluster can be recommended for the rain-fed condition under organic farming system.

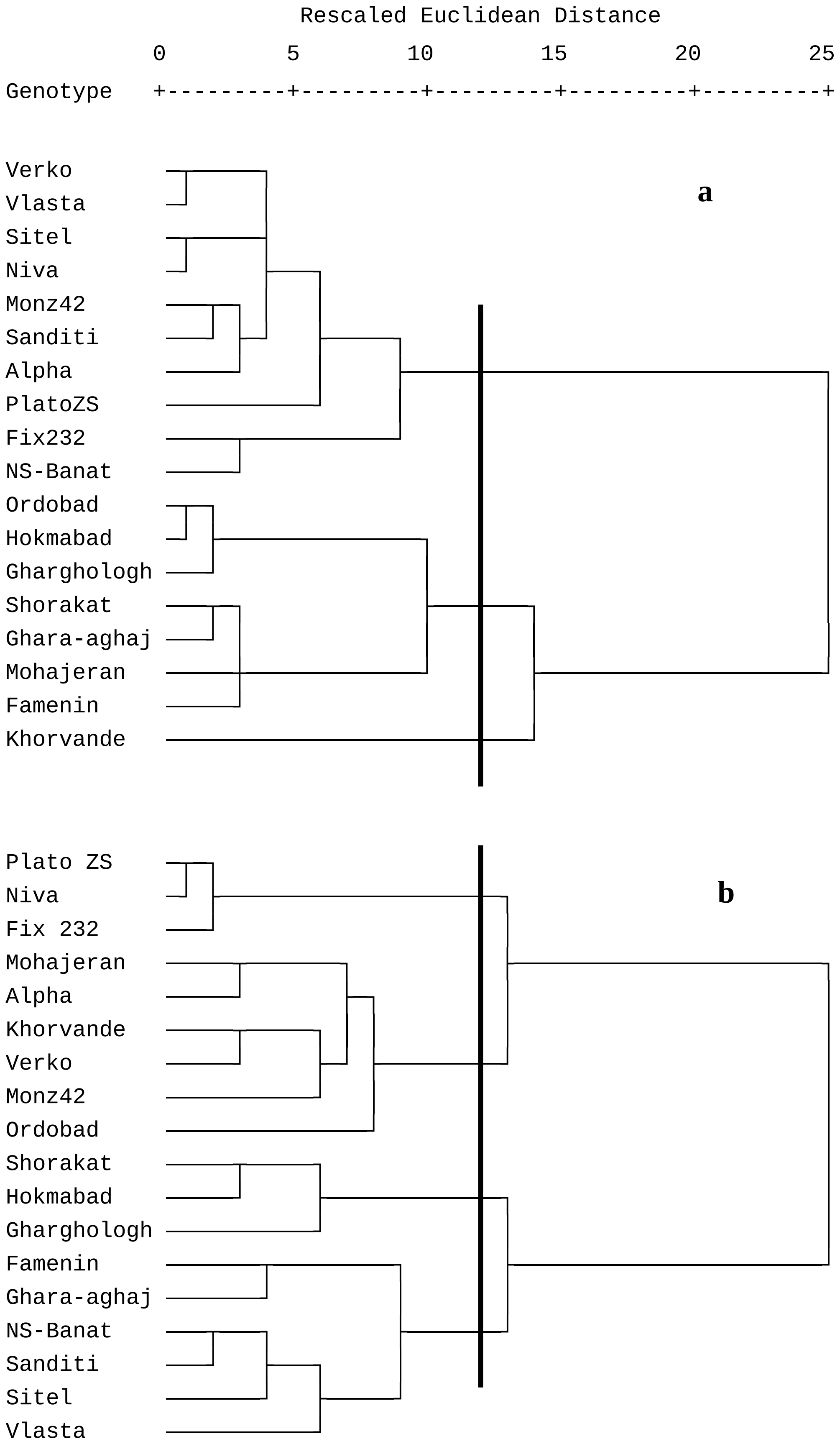


Fig. 11. Continued

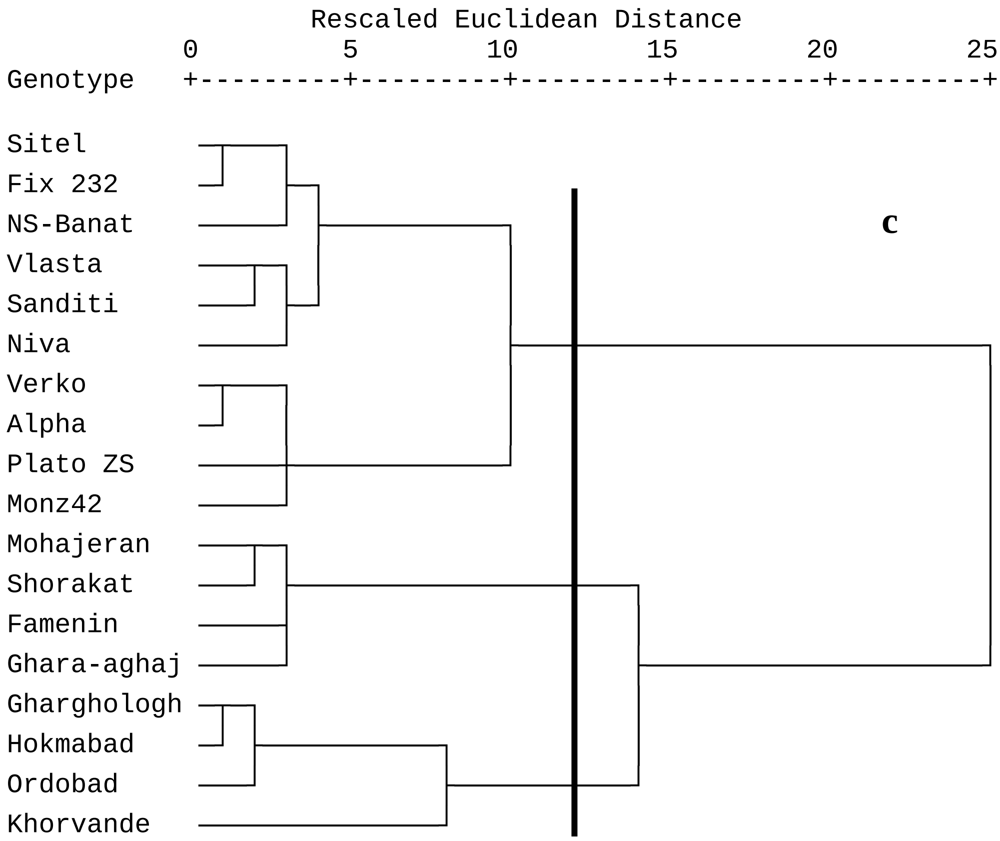


Fig. 11. Dendrogram of cluster analysis for 18 alfalfa genotypes using Ward method under irrigated (a), rain-fed (b) and average over two conditions (c).

Based on average values across two conditions, genotypes can be classified into 3 clusters (Fig 11c and Table 17). The first cluster included 4 Iranian ecotypes (Mohajeran, Famenin, Shorakat and Ghara-aghaj) which are described by rapid crop regrowth, tall stem, more stem number per m^2, relatively high shoot dry matter but less LAI, leaf to stem ratio and shoot protein content. The second cluster comprised the 4 remainder of Iranian ecotypes (Ghargholigh, Hokmabad, Ordobad and Khorvande) which can be defined only by high root dry matter. The third cluster contained all European cultivars (Sitel, Verko, Vlasta, Monz42, Fix 232, NS-Banat, Sanditi, Alpha, Plato ZS, and Niva) that were characterized by high shoot dry matter and protein content and relatively tall stems but crop regrowth, stem number per m^2 and root dry matter less than the total mean. With regard to variation within each cluster, genotypes in cluster 1 or 3 can be recommended and used for wider adaptation and farming condition.

Finally, in non-stressed condition a cluster analysis based on the studied characters was able to clearly differentiate Iranian ecotypes and European cultivars, whereas drought stress effects under rain-fed condition and different responses of genotypes caused changing of classification. The result of classification based on average values over two conditions was more and less similar to irrigated condition.

Table 16. Genetic distance among alfalfa genotypes calculated by morphological and physiological data under different conditions.

Genotype		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
2	IR	7.4																
	RN	3.0																
	AVE	5.8																
3	IR	2.6	5.6															
	RN	4.4	5.4															
	AVE	2.8	6.0															
4	IR	4.4	4.3	2.6														
	RN	2.6	4.0	3.7														
	AVE	3.4	4.4	3.3														
5	IR	3.6	5.3	2.7	2.1													
	RN	3.8	3.3	4.5	4.2													
	AVE	3.3	3.7	3.5	2.4													
6	IR	2.1	6.5	2.4	3.6	2.7												
	RN	3.7	5.1	3.3	3.3	4.6												
	AVE	2.0	5.9	2.6	3.0	3.6												
7	IR	2.5	6.6	2.5	3.3	2.3	1.9											
	RN	5.2	6.8	2.7	4.7	6.6	3.6											
	AVE	2.4	7.3	2.5	4.4	4.7	2.4											
8	IR	4.4	4.8	3.1	1.9	1.5	3.5	2.9										
	RN	2.8	3.8	2.8	3.1	3.7	2.2	3.8										
	AVE	2.6	4.2	3.2	1.6	1.6	2.7	3.9										
9	IR	4.4	6.1	4.3	3.8	3.7	4.4	3.7	3.9									
	RN	4.9	5.3	3.5	4.8	5.8	4.3	3.3	4.3									
	AVE	3.5	6.4	4.0	4.8	4.2	4.1	3.9	4.4									
10	IR	4.2	6.8	4.2	3.8	2.9	3.9	3.0	3.4	2.0								
	RN	3.3	2.2	4.3	3.9	3.2	4.8	5.8	3.9	3.8								
	AVE	3.6	5.4	4.6	4.2	3.3	4.7	4.6	3.6	2.4								
11	IR	4.5	6.8	4.5	4.0	3.0	4.2	2.9	3.3	2.2	1.3							
	RN	5.1	6.5	4.6	5.4	6.7	3.2	3.2	3.9	3.7	5.9							
	AVE	2.8	6.5	4.4	4.5	4.1	3.2	3.3	3.7	2.4	3.0							
12	IR	4.9	7.1	4.4	3.7	3.7	4.6	3.4	3.8	2.4	2.1	2.3						
	RN	4.1	3.6	4.0	4.5	4.5	4.0	4.9	3.5	3.1	2.6	4.7						
	AVE	4.4	6.3	4.7	4.6	4.0	4.9	4.5	4.0	2.8	2.2	2.9						
13	IR	4.4	6.8	4.0	3.5	3.5	4.3	4.0	4.3	2.4	2.5	3.3	2.7					
	RN	3.8	3.8	4.4	5.1	4.7	5.0	5.0	3.9	3.0	2.5	4.7	2.7					
	AVE	3.6	5.9	4.5	4.4	3.9	4.1	4.2	3.9	1.3	2.1	1.9	2.1					
14	IR	5.1	5.0	4.5	3.3	3.8	4.9	4.7	4.1	2.2	3.5	3.6	3.7	2.6				
	RN	3.4	4.3	2.9	4.1	4.6	3.5	3.3	2.6	2.5	3.3	3.3	3.2	2.2				
	AVE	3.3	4.7	4.1	4.0	3.3	3.3	4.4	3.4	2.5	3.3	2.7	3.8	2.2				
15	IR	4.4	6.6	4.2	4.0	3.9	4.3	2.9	4.0	2.2	2.6	2.2	1.9	3.5	3.7			
	RN	4.3	5.6	3.8	5.1	5.9	3.7	3.3	3.7	2.6	4.5	2.4	3.4	2.8	2.1			
	AVE	3.2	6.5	4.3	5.2	4.3	4.1	3.5	4.1	2.2	2.8	1.9	2.6	2.2	3.1			
15	IR	4.2	7.1	3.9	4.3	3.8	4.5	3.1	4.2	2.6	2.2	2.3	2.5	3.2	4.2	2.3		
	RN	2.2	3.0	4.7	3.9	4.4	4.8	5.7	3.8	4.6	2.6	5.7	3.3	2.9	3.4	4.0		
	AVE	3.7	6.0	4.8	4.9	4.0	5.1	4.6	4.1	3.5	1.8	3.6	2.9	3.4	4.4	3.0		
17	IR	6.3	8.0	6.2	5.5	4.8	6.3	4.9	4.6	3.7	2.8	2.5	3.3	4.7	5.2	3.8	3.2	
	RN	3.3	3.9	4.3	4.6	4.1	4.1	5.0	3.6	3.3	2.7	4.2	2.7	1.8	2.6	2.7	3.1	
	AVE	5.0	7.0	6.3	5.9	4.7	6.3	6.0	5.1	3.9	2.4	3.9	3.0	3.5	5.0	3.6	2.7	
18	IR	4.0	5.7	3.5	3.3	3.2	3.8	2.9	3.1	1.7	2.3	2.3	2.6	3.5	3.3	2.0	2.5	3.7
	RN	3.7	4.5	3.9	4.9	4.5	4.1	4.3	3.4	2.9	3.3	3.6	3.1	1.6	1.9	2.1	3.6	1.3
	AVE	3.3	5.6	4.4	4.8	3.5	4.4	4.4	3.8	2.3	2.2	2.9	3.0	2.3	2.8	2.0	3.1	3.2

Number 1 to 18 are genotypes Mohajeran, Khorvande Famenin, Gharghologh, Ordobad, Shorakat, Ghara-aghaj, Hokmabad, Sitel, Verko, Vlasta, Monz 42, Fix 232,NS-Banat , Sanditi, Alpha, Plato ZS and Niva, respectively.

Table 17. Mean, number of members (N) and standard deviation of the resulting clusters based on studied traits under different conditions.

Irrigated

Cluster	Trait	CR	PH	STN	LAI	LSR	SHDM	RODM	SHCP
1	Mean	29.4	86.9	1174.3	4.3	0.6	15.6	8.6	22.3
	N	7	7	7	7	7	7	7	7
	Std. Deviation	1.6	2.3	44.3	0.1	0.03	1.5	1.2	0.6
2	Mean	33.2	77.5	1016.6	3.7	0.7	11.5	10.2	22.5
	N	1	1	1	1	1	1	1	1
	Std. Deviation	-	-	-	-	-	-	-	-
3	Mean	27.6	86.6	1059.2	4.6	0.8	16.6	7.8	22.9
	N	10	10	10	10	10	10	10	10
	Std. Deviation	2.4	1.9	48.8	0.2	0.02	0.7	1.6	0.5
Total	Mean	28.6	86.2	1101.6	4.4	0.7	15.9	8.3	22.6
	N	18	18	18	18	18	18	18	18
	Std. Deviation	2.4	2.9	74.9	0.3	0.1	1.6	1.5	0.6

Rain-fed

Cluster	Trait	CR	PH	STN	LAI	LSR	SHDM	RODM	SHCP
1	Mean	17.7	60.5	924.6	2.4	0.9	9.0	6.5	23.2
	N	6	6	6	6	6	6	6	6
	Std. Deviation	0.3	1.6	101.2	0.3	0.1	0.8	0.6	0.5
2	Mean	19.8	66.0	999.9	2.7	0.8	11.4	7.0	22.3
	N	6	6	6	6	6	6	6	6
	Std. Deviation	0.8	1.9	53.4	0.1	0.1	0.7	1.0	0.9
3	Mean	18.6	61.8	1017.2	2.4	0.8	9.3	8.3	22.4
	N	3	3	3	3	3	3	3	3
	Std. Deviation	1.5	1.5	46.0	0.2	0.0	0.8	0.7	0.2
4	Mean	18.8	65.3	870.8	2.6	0.9	10.8	6.2	23.7
	N	3	3	3	3	3	3	3	3
	Std. Deviation	0.5	1.1	11.8	0.0	0.0	0.3	0.6	0.3
Total	Mean	18.7	63.3	956.2	2.5	0.8	10.1	6.9	22.8
	N	18	18	18	18	18	18	18	18
	Std. Deviation	1.2	2.9	84.2	0.2	0.1	1.3	1.0	0.8

Average over two locations

Cluster	Trait	CR	PH	STN	LAI	LSR	SHDM	RODM	SHCP
1	Mean	24.8	76.1	1110.6	3.4	0.7	13.3	7.6	22.1
	N	4	4	4	4	4	4	4	4
	Std. Deviation	0.6	1.7	32.2	0.1	0.0	0.5	0.7	0.6
2	Mean	23.7	71.6	1024.7	3.2	0.8	11.1	8.6	22.7
	N	4	4	4	4	4	4	4	4
	Std. Deviation	1.1	2.3	61.7	0.1	0.1	1.0	0.5	0.3
3	Mean	23.3	75.5	997.9	3.6	0.8	13.6	7.2	23.0
	N	10	10	10	10	10	10	10	10
	Std. Deviation	1.6	1.6	38.6	0.1	0.0	0.7	0.8	0.4
Total	Mean	23.7	74.8	1028.9	3.5	0.8	13.0	7.6	22.8
	N	18	18	18	18	18	18	18	18
	Std. Deviation	1.4	2.4	61.5	0.2	0.1	1.3	0.9	0.6

CR= Crop regrowth; PH= Plant height; STN= Stem number per m^2; LAI= Leaf area index; LSR= Leaf to stem ratio; SDHM= Shoot dry matter; RODM= Root dry matter; SHCP= Shoot protein content

The ecotypes Hokmabad and Ghargholoh have been classified in same cluster in all cases as well as Famenin and Ghara-aghaj, indicating high their genetic similarity based on studied traits.

Genetic distances (Euclidean distance) calculated by morphological data in addition to shoot DM, shoot protein content and root DM among tested genotypes are displayed in Table 16. A smaller value of distance shows lower genetic diversity or more genetic similarity. Genetic diversity among Iranian ecotypes was higher than that of European cultivars (Table 16). It can be owing to wider genetic base of tested ecotypes compared to registered European cultivars. Among Iranian ecotypes, Khorvande had the highest genetic distance from other genotypes as it was grouped in separate cluster under irrigated condition (Figure 11). In irrigated trial, the most similar pair of genotypes were Verko and Vlasta followed by Ordobad and Hokmabad and then Sitel and Niva. Under rain-fed condition, the most similar genotypes were Plato ZS, Niva and Fix232 followed by NS-Banat and Sanditi and then Khorvande and Verko. With regard to average values over two conditions, Sitel and Fix232, Ghargholoh and Hokmabad, and Verko and Alpha were the most similar genotype pairs, respectively.

Discussion

The significant difference between the two locations, irrigated and rain-fed (Table 3), concerning most of the studied characters showed an effect of water stress on crop performance, plant morphological and agronomical characters and reflects the diverse available water for plant roots due to different water supply and water holding capacity in the two conditions. The reduction in shoot dry matter confirms previous research on the influence of water deficit on alfalfa production (Carter and Sheaffer, 1983; Grimes *et al.*, 1992; Brown *et al.*, 2005; Pembleton *et al.*, 2009).

Non-significant change in average of node number per stem under rain-fed condition indicates shorter internodes in drought condition with regard to reduction of plant height (Table 5). Pembleton *et al.* (2009) reported a reduction of shoot elongation under water deficit leading to shorter shoots and internodes. Brown and Tanner (1983) found that shoot density was reduced by 77 % under drought stress in the early regrowth period.

The reduction of LAI and stem number per m^2 indicated less density and consequently more penetration of sunlight and evaporation under rain-fed rather than irrigated condition. Pembleton *et al.* (2009) also reported significant effects of water deficit on shoots per plant and shoot mass. However, crop characters were affected differently by drought conditions. While average of leaf to stem ratio, shoot and root protein content increased, the economically important characters such as shoot dry matter and BNF were reduced under drought condition (Table 5). An increase in leaf to stem ratio under drought stress was also reported in other studies (Carter and Sheaffer, 1983; Halim *et al.,* 1989b; Pembleton *et al.,* 2009). Mild water deficit during early vegetative growth caused a greater reduction in stem production than in leaf production, leading to a higher leaf-to-stem ratio when water applications ceased after 2 wk of regrowth (Halim *et al.*, 1989a).

Shoot and root protein content increased slightly under rain-fed condition. Although an alfalfa field may appear as a uniform mass of green, the harvested crop is made up of stems, leaves, flowers and petioles, and each part differs in nutritional value. The most important of these by weight are stems and leaves. Leaves are much more digestible and lower in fiber than stems, and can have 2 to 3 times more crude protein (CP) content than stems. Thus, the relative weight of leaves and stems is an important visual determinant of quality for alfalfa (Robinson *et al.* 2007). Additionally, considering protein content as main component and indicator of forage quality (Rotili *et al.* 2004) and relationship between leaf to stem ratio and shoot protein content (Table 12; Scotti *et al.* 2006), it can be concluded that forage quality increased under drought stress condition, while forage quantity and biomass production decreased.

The comparison between reduction of shoot dry matter and root dry matter (Table 5) specified that above ground biomass was more sensitive to drought condition than below ground biomass.

Significant difference among genotypes for all characters, except for node number per stem and chlorophyll content, exhibited high genetic variation among tested materials. Diverse geographical conditions from which the tested genotypes originated or where they were improved can be regarded as the main reason for the high genetic variation and indicate different gene pools. Although there were differences within Iranian or European genotypes, main reason of the meaningful differences among genotypes for diverse traits originated in difference between Iranian and European genotypes. This can be justified by results of contrast equations between these two groups for different traits (Tables 3 and 6). It shows different adaptation and selection pattern of Iranian and European genotypes. Iranian ecotypes, naturally selected under dry conditions, exhibited more root biomass and root protein content and lower shoot dry matter and forage quality (shoot protein content and leaf to stem ratio) than European cultivars (Table 6).

There are different ideas about the role of root biomass and development in suitability of genotypes for drought-prone environment. Some considered root development as an important indicator for estimating suitability to drought resistance (Lu 1992; Ren 1998) and strong root development as a characteristic of lucerne cultivars adapted to dryland conditions (Guo *et al.* 2002). On the contrary, Annicchiarico (2007) stated an extensive root is not necessarily related to adaptation to drought-prone environments. Johnson and Tieszen (1994) found that greater photosynthate partitioning to roots was associated with lower shoot water-use efficiency. Based on our results, some of the genotypes with high root dry matter showed low shoot dry matter such as Khorvande and Ghargholog or Iranian ecotypes vs. European cultivars (Table 6). However, it seems that it depends on genotype. For instance, Vlasta, Fix232 and NS-Banat had root and shoot dry matter more than grand mean. Therefore, root yield can be considered as a selection criterion as well as shoot yield to recommend suitable cultivar in organic systems especially for forage use management in drought-prone environment.

Significant genotype by environment (GE) interaction (GL, GY and GLY in Table 3) has been found for different traits, especially for biomass production characters (like shoot dry matter and total biomass yield) and its components (like plant height and stem number per m^2) indicating changing of performances and ranks of genotypes from irrigated to rain-fed condition or from first to second year. The ecotype Ordobad had the least number of stems per m^2 (773.2) at rain-fed condition while this ecotype had the second rank (1207.6) under irrigated condition (Table 7). The ecotype Mohajeran which had the highest shoot dry matter yield (18.45 t h^{-1}) at irrigated condition, showed a yield (9.40 t h^{-1}) less than the mean yield of genotypes under rain-fed conditions (Table 7). While root dry matter of cultivar Fix232 was the lowest (5.54 t h^{-1}) at rain-fed condition, its root yield at irrigated condition was grouped in the best (Table 7). It seems that stress condition has not allowed some genotypes to express their genetic potential. However, the performance or rank changes were differed by trait under consideration and genotype which indicated there was variability in the effect of environmental conditions like water supply for a range of genotypes and traits. On the other hand, some genotypes were affected less than others by environmental factors such as drought stress under rain-fed condition (Table 7). While Ghargholog, NS-Banat and Sanditi showed -3 % reductions in stem number per m^2, Ordobad decreased -36 % under rain-fed condition. Concerning leaf to stem ratio, Mohajeran with +48 % and NS-Banat with +11% had the highest and lowest increase due to drought stress under rain-fed condition. Mohajeran with -49 % for shoot dry matter and Fix232 with -47, -41 and -39 % for root dry matter, total biomass yield and BNF had the greatest reduction and changes. Annicchiarico (1992) and Annicchiarico and Piano (2005) reported a highly significant GL interaction for dry matter yield of alfalfa cultivars in northern Italy. Cross-over interaction has been detected between top-ranking materials at contrasting locations (Annicchiarico 1992). We found cross-over interaction only for root dry matter and shoot protein content. Regarding to significant GL interaction and its type and performance stability of genotypes in different conditions, selection among materials can be done for wide or specific adaptation, i.e. selection of the best genotype for each environment or one widely adapted genotype for all environments.

In addition, regarding the forage utilization system- forage use as feeding source or mulching as green manure- selected genotypes can differ due to considering different properties at the same time. The non-harvestable biomass yield (stubble and root) plays an important role to supply N for subsequent crops in a rotation in organic system when shoot biomass is removed from field. In drought-prone environment, in particular arid and semi-arid conditions, harvested forage of legume crop is the main source of protein and energy for livestock. Hoeppner *et al.* (2006) expressed that the energy efficiency and subsequently the sustainability of organic farming systems may be increased by including alfalfa in the crop rotations. Therefore, in this study, we considered performance and its stability of 3 important characters of genotypes; shoot dry matter, total biomass yield and BNF; to select and recommend best genotype/ genotypes for each condition. These characters regarded above and below ground productivity as well as legume-rhizobium symbiosis potentials for genotypes at same time.

In selection of genotypes with specific adaptation to each condition, the AMMI analysis was the suitable method due to analyzing information only for 2 years. Despite of significant GL interaction, a stable and high yielding genotype (widely adapted genotype) for both conditions (no crossover interaction among top yielding cultivar) was selected. Although the number of environments was small, the selection only based on genotype performance showed different results rather than simultaneous selection along with stability (Tables 8 and 10). In addition, selected genotypes based on different stability parameters and characters were different. Incorporation of all information in a single measure by adding up of ranks of a genotype in different situations facilitated the selection of suitable genotypes as well as using of all advantages and discarding of weakness of different stability parameters. In this study, the cultivar Sitel proved as widely adapted cultivar followed by PlatoZS, NS-Banat, Vlasta and Ghargholog h. European genotypes bred and adopted to Europe mega-environment may have performance and stability advantages in Europe rather than Iranian ones.

Based on amount of rainfall and its distribution at first and second year (Fig. 1) and average yield and IPCA scores (Figs. 4 and 5) of four environments, the intensity of drought stress at first year, particularly under rain-fed trial, was more than second year, indicating that more stress intensity was accompanied by more variability among genotypes and increased GE interaction. On the contrary, in the second year of study with improvement of distribution pattern of rainfall and reduction of stress intensity, RS and GS (Second year of rain-fed and irrigated trials) had less effect on GE interaction.

High correlation between $б_i^2$ and ASV (Table 11) and agreement of ASV and AMMI2 biplot suggesting that using one of them is sufficient based on researcher choice and goal of experiment. Fikere *et al.* (2008) also found significant positive rank correlation between ASV and $б_i^2$ (r = 0.818**) in faba bean. Although AMMI was found to be more informative in describing the adaptive response of the genotypes, with regard to correlation between different stability parameters and crop performance (Table 11), P_i was the best stability parameter to select high yield and stable genotype based on simplicity of calculation and correlation with crop performance in this study.

The acknowledging of correlations between traits is a fundamental subject in plant breeding programs. Breeders make effort to develop suitable new cultivars by selection and determination of suitable selection criteria for different environment conditions to find high yielding cultivars more easily. Selection for a certain trait according to phenotypic expression may change other traits at the same time. Recognition of correlation provides the possibility of simultaneous improvement of more traits and is also important for traits of low genetic variability or heritability, in cases of which the progress in selection is achieved by indirect selection and methods. The correlations between some characters were condition-specific. For example, the correlation between crop regrowth and shoot dry matter and its important component, plant height, was opposite under irrigated and rain-fed condition (Table 12). It showed that genotypes with rapid crop regrowth in stress condition may avoid from stress effects and finally can produce more dry matter yield. On the contrary, under irrigated and optimum condition, genotype/genotypes with rapid crop regrowth, maybe because they don’t use the whole growth period, can’t produce maximum dry matter yield. Under rain-fed condition, the genotypes Vlasta, Sanditi and Ghara-aghaj with rapid crop regrowth had the greatest plant height and LAI and shoot DM yield (after Sitel) (Table 7). The genotype Khorvande which had first rank of crop regrowth rate under irrigated condition, regarding to plant height, LAI, and shoot DM yield was on the last rank (Table 7). The positive correlation between root dry matter and crop regrowth in both conditions and negative correlation between root DM and shoot DM can explained the reason of rapid regrowth of some genotypes under irrigated condition. Johnson and Tieszen (1994) found that greater photosynthate partitioning to roots was associated with lower shoot water-use efficiency.

Annicchiarico (2007) found that higher root biomass in some alfalfa genotypes was associated with somewhat lower aerial DM over harvests, higher ratio of root DM to total DM, and a trend towards lower plant mortality and less stems per plant. Khorvande with high root biomass showed the lowest shoot DM under irrigated condition. Specifically adapted cultivars to no stress condition show greater root DM, resulting in more root nitrogen reserves by means of larger taproots and greater nitrogen concentration and consequently faster shoot regrowth (Rotili *et al*., 1994; Avice *et al*., 1997a; Annicchiarico 2007). In this study, Fix 232 with high shoot dry matter had high root DM, while Mohajeran with the highest shoot DM didn't have high root biomass (Table 6).

Regarding to correlations among plant height, LAI, Shoot DM and total biomass yield (Table 11), it may be concluded that the high yielding genotypes had higher plants and denser stands, especially under rain-fed condition. Phenotypic correlations in alfalfa, specifically *sativa*, generally indicate higher yielding plants will be taller, more mature, and have reduced nutritive value (Elliot *et al*., 1972). Riday and Brummer (2005) reported significant positive correlation between plant height and crop spring, midseason and autumn regrowth. Riday and Brummer (2005) found a significant and negative correlation between spring regrowth and crude protein (r = -0.30*) and a highly significant positive correlation between leaf to stem ratio and midseason (r = 0.39***) and autumn (r = 0.42***) regrowth. Riday and Brummer (2005) found significant and positive correlation between plant height and dry matter yield. Tucak *et al*. (2008) found strong positive correlation between dry matter yield and plant height (r = 0.87**), negligible negative correlation between dry matter yield and protein content (r = -0.04) and negative significance correlation between dry matter and leaf ratio associated to 12 genotypes in 18 environments. Rosellini and Veronesi (2006) reported positive and significant relations between yield and its components (height, number of stems, regeneration). Julier *et al.* (2000) found significantly negative phenotypic and genotypic correlations between dry matter yield and leaf to stem ration (r = -0.47** and -0.37**, respectively) across harvest dates in 56 alfalfa cultivars.

Correlation coefficient which measures the simple linear relationship between two traits does not predict the success of selection. However, path analyses, regression on standardized variables, determines the relative importance of direct and indirect effects of traits on a dependent trait like BNF or shoot DM. Path analysis was developed as a method of decomposing correlations into different pieces (direct and indirect effects) for interpretation of effects. Path coefficient analyses are more informative and useful than simple correlation coefficients and widely used in crop breeding to determine the nature of relationships between yield and some yield components (Dewey and Lu, 1959; Kang *et al.*, 1983; Williams *et al.*, 1990; Gravois and McNew, 1993; Board *et al.*, 1997; Samonte *et al.*, 1998). All direct effects of BNF components were positive in both conditions, while some of indirect effects were negative (Table 13). Different negative indirect effects of these main characters on BNF via each others or other characters in the model impede the BNF improvement. The selection procedure should be formulated so that the advance in one component is not jeopardized by the deterioration effect of the other (Izge *et al*. 2006). Under these circumstances, a restricted simultaneous selection model is to be followed, i.e. restrictions are to be imposed to nullify the undesirable indirect effects to make use of the direct effect (Singh and Chaudhary, 1977).

Direct effects of root protein content in both conditions and root DM under irrigated trial were reduced by large negative indirect effect via shoot DM, because of a high negative correlation between these characters especially under irrigated condition (Table 12). The negative indirect effects of root DM and protein content via other characters and components on BNF in the model, ultimately, caused significant negative correlation between root protein content and BNF, and insignificant positive correlation between root DM and BNF. Regarding importance of BNF in organic farming systems and additionally, the role of non-harvestable parts of alfalfa crop, i.e. root and stubble, in forage use management, indirect improvement of BNF via proper selection criteria which consider negative correlations between components of criteria are necessary and inevitable.

With regard to results of path analysis and positive cooperation in both conditions (Table 14), plant height and LAI can be considered as primary selection criteria for improving shoot DM. It can also be deduced from genotype means for different characters (Table 7) that the high yielding genotypes had higher plants and denser stands, especially under rain-fed condition. Rosellini and Veronesi (2006) reported a strong direct effect of height on yield (0.57**) in a panonian alfalfa ecotype. Kephart *et al.* (1992) found that shoot weight was the major influence on alfalfa yield in different seed rates at two locations. The path analysis for forage yield in 30 polycross progenies of alfalfa indicated that the most effective traits on fresh and dry forage yields were plant height and number of shoots, respectively (Monirifar *et al.*,2003). Crop regrowth and plant height can be considered as primary selection criteria, with antonymous effects, for improving root dry matter. With regard to importance of plant height in indirect selection for improving shoot DM and consequently BNF, selection must be done for taller and rapidly regrowing individuals and genotypes. The cultivars Vlasta and NS-Banat showed relatively high shoot and root DM due to suitable average of plant height, crop regrowth and LAI over two conditions (Table 6).

Under irrigated condition and on the average of two conditions, cluster analysis clearly differentiated Iranian ecotypes and European cultivars from each others (Figs. 11a and c), whereas under rain-fed condition, influence of drought stress and different response of genotypes resulted in small change in grouping of genotypes (Fig. 11b). Herbert *et al.* (1994) observed more variability among annual medic due to increasing environmental stresses. Dehghan-Shoar *et al.* (2005) reported that plant morphological characters had insufficient usefulness in identification and discrimination of Iranian and New Zealand alfalfa cultivars. Tucak *et al*., (2008) reported that morphological characters were not sufficient to determine the differences among alfalfa cultivars and populations. Different tested genotypes, environment and their interaction in addition to different characters used in the study can be possible explanations for contrasting results of this study.

The genetic distance, based on characters used in cluster analysis, between Iranian and European genotypes was higher than of that within Iranian or European genotypes. However, genetic distance and variability within Iranian ecotypes was higher than in European improved cultivars. Characters used in the cluster analysis and calculation of genetic distances cover the most important agronomical genes in alfalfa genome. So, different clusters and more genetic distances represent different gene pool and alleles of measured traits in dissimilar genotypes. Genetic diversity of initial selection materials is essential for successful breeding and creation of new cultivars. With respect to importance of alfalfa ecotypes in breeding programs and suitable genetic distance between Iranian ecotypes such as Khorvande and Mohajeran with European cultivars, crossing program between diverse genotypes can be planned to construct new varieties especially for organic farming systems. Bauchan *et al.* (1993) selected a core collection to use in breeding programs after evaluation and classification of 122 alfalfa genotypes by cluster analysis.

Finally, based on objectives of this study, it can be concluded that:

1. There were significant differences between years, locations, genotypes and their interactions in the most of studied characters. Drought stress condition in rain-fed trial reduced the average of all traits except for leaf to stem ratio, shoot and root protein content. The cultivar Sitel was the best genotype (as wide adapted genotype) regarding to mean comparisons and stability analysis for shoot dry matter, total biomass yield and BNF, followed by PlatoZS, NS-Banat, Vlasta and Ghargholog h. As specific adaptation, Sitel, Fix232, Mohajeran, NS-Banat and Verko were suitable for irrigated condition, respectively. Vlasta, Sitel, Ghara-aghaj, Niva and Sanditi were suitable under rain-fed condition, respectively.

2. Although, AMMI analysis was found to be more informative in describing the adaptive response of the genotypes, P_i (superiority measure) was the best stability parameter to select high yield and stable genotype based on simplicity of calculation and correlation with crop performance in this study.
3. Regarding to correlation coefficients, it is concluded that the high yielding genotypes had taller plants and denser stands, especially under rain-fed condition. In path analysis, all direct effects of BNF components were positive in both conditions, while some of indirect effects were negative. Plant height and LAI can be considered as primary selection criteria for improving shoot DM, while crop regrowth and plant height, with antonymous effects, were more important for improving root dry matter.
4. Cluster analysis clearly differentiated Iranian ecotypes and European cultivars from each others using morphological and physiological data. However, under rain-fed condition, influence of drought stress and different response of genotypes resulted in small change in grouping of genotypes. Regarding to considerable genetic distance between Iranian and European genotypes in this study, different crossing program can be planned between diverse genotypes to construct new varieties especially for organic farming systems.

Chapter Two

Suitability of Different Drought Tolerance Criteria and Alfalfa (*Medicago sativa* L.) Genotypes under Different Conditions of Organic Farming

Suitability of Different Drought Tolerance Criteria and Alfalfa Genotypes under Different Conditions of Organic Farming

Abstract

Alfalfa is usually grown as a rain-fed crop in crop rotations in organic farming systems in eastern Austria, where year-to-year fluctuations of rainfall cause different levels of drought stress. In order to identify the suitability of different alfalfa genotypes and drought tolerance indices, 18 alfalfa genotypes were evaluated under irrigated and rain-fed conditions at the research station of the University of Natural Resources and Applied Life Sciences (BOKU), Vienna, Austria, during 2007-08. Five drought tolerance selection indices- tolerance (TOL), mean productivity (MP), geometric mean productivity (GMP), stress susceptibility index (SSI) and stress tolerance index (STI) along with superiority statistic (P_i)- were calculated using shoot dry matter, total biomass yield and biological nitrogen fixation (BNF) data. The first year, the average over two years and the second year of study were considered as high, medium and low stress intensity (SI) conditions, respectively. The association between irrigated and rain-fed performances increased with decreasing stress intensity (SI) from the first to the second study year. TOL and SSI showed high correlation only with rain-fed performance while other indices correlated significantly with both conditions. Sitel, Plato ZS, Vlasta and NS-Banat were the best genotypes based on their performances in both conditions. STI and GMP were the best indices to distinguish genotypes with high performance in both conditions, while SSI was the best one to separate genotypes with high yield potential under rain-fed condition.

Keywords: Lucerne, Iranian ecotypes, Shoot dry matter, Biological nitrogen fixation, Total biomass yield.

Introduction

Legume fodder crops such as alfalfa (*Medicago sativa* L.) are an essential component of organic farming systems, especially under arid and semiarid conditions. The energy efficiency and the sustainability of such systems can be increased by including alfalfa in the crop rotations (Hoeppner *et al.*,2006). Stockless organic farming is predominant in the dry, pannonian region of eastern Austria, and alfalfa – with its high biological nitrogen fixation (BNF) ability and drought tolerance – is the best known fodder crop in this region. Alfalfa can play an important role in the crop-livestock organic systems of southern Europe, because of its adaptation to low input or drought stressed conditions, its positive effect on the nitrogen balance and the soil fertility, and the high protein content and quality of its forage (Campiglia *et al*., 1999; Huyghe, 2003; Annicchiarico *et al*., 2006).

Alfalfa is usually grown as a rain-fed crop in crop rotations here, where the amount, frequency and duration of rainfall fluctuate from year-to-year. The effect of drought stress is enhanced when accompanied by high temperature, biotic stresses and undesirable soil characteristics such as low water-holding capacity. Selection under solely favorable or stress conditions may lead to specifically adapted genotypes with a suitable response under these specific conditions. Ceccarelli and Grando (1991) stated that breeding programs can produce cultivars with contrasting adaptation patterns by adopting distinct genetic bases (each including material with the desired adaptive response), distinct selection environments (each representative of the target population in a particular environment), or both. Thus, there are three approaches to select breeding strategy for stress environments. Some researchers believe in selection under good conditions and subsequent yield testing in stress environments (Roy and Murty 1970; Mederksi and Jeffers 1973; Richards 1996; Betran *et al.,* 2003). They assume that superior genotypes in favorable conditions will also provide relatively good yields in stress conditions, and also that genotypes selected in stress conditions will show a low yield potential in good environments (Ceccarelli 1987). Followers of the second approach rely on direct selection in target stress conditions (Boyer and McPherson 1975; Johnson 1980; Buddenhagen 1983; Ceccarelli 1987; Ceccarelli and Grando 1991). They believe that direct selection is most efficient in increasing yield in the target stress condition. Accordingly, direct selection in stress environments will decrease yield in non-stress environments unless genetic variances in stress environments are considerably greater than those in non-stress environments and unless genetic correlations between them are positive and close to 1 (Rosielle and Hamblin 1981). Based on the specific strengths and weaknesses of the two above-mentioned approaches, the simultaneous selection under stress and non-stress conditions seems to be more logical and confidence inspiring. Based on this, some researchers believe in a third approach: selection under favorable and unfavorable conditions at same time (Fischer and Maurer 1978; Clark *et al.*, 1992: Fernandez 1992).

Based on their performance in stress and non-stress environments, Fernandez (1992) categorized genotypes into four groups: Group A contains genotypes with uniform superiority in both stress and non-stress environments; Group B includes genotypes that perform favorably only in non-stress environments; Group C are genotypes that have relatively high yields only in stress environments; and Group D contains genotypes with poor performance in both stress and non-stress environments. Several selection criteria are proposed to select genotypes based on the mathematical relationship between their performance in stress and non-stress conditions (Rosielle and Hamblin 1981; Fischer and Maurer 1987; Fernandez 1992). The optimal selection index and criterion should distinguish Group A (widely adapted genotypes) from the other three groups.

Forage yield trials in two contrasting conditions; non-stress and stress are widely used to select suitable and adapted genotypes to both environments. In addition to shoot yield (harvestable biomass), the yield of non-harvestable biomass (stubble and root) can play an important role in total BNF and in supplying N for following crop, especially in forage use management (as organic dairy feed) of organic systems. In a forage use management, most of the fixed N_2 is removed by harvesting the forage legumes, reducing the benefit to the subsequent crops (Pietsch *et al.*, 2007). However, 30-60 % of the legume's total plant N may be below-ground associated with roots and nodules (Peoples *et al.* 2009). Consequently, crop legume residues can still contain considerable fixed N even after a large amount of N is removed at harvest. Therefore, considering total biomass yield and BNF along with shoot dry matter can help select genotypes that are superior in crop rotations. The objectives of this study were 1) to identify widely adapted and the most tolerant genotypes among 18 alfalfa genotypes based on important economic and ecological traits; and 2) to evaluate various drought tolerant indices and identify the best one under different stress intensity.

Materials and Methods

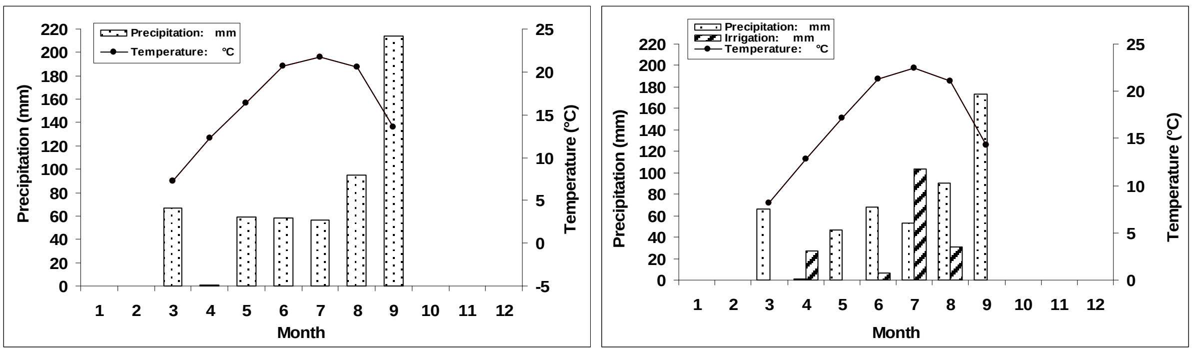
Site and experiment description

In order to identify the suitability of different alfalfa genotypes and drought tolerance indices, this study was carried out in two separate trials, namely irrigated (no water stress) and rain-fed (water stress) at two different organically managed fields, Gross-Enzersdorf (48º12' N, 16º33' E) and Raasdorf (48º15' N, 16º37' E), respectively. Both field are located at research stations of the University of Natural Resources and Applied Life Sciences (BOKU), Vienna, Austria. The farm managements were organic, stockless and no organic manures were applied. The soils are Calcaric Phaeozems (WRB) from loess with a silty loam texture. Some properties of soils are shown in Table 1. The soils are described in detail in Freyer *et al.*, (2000) and Pietsch *et al.*, (2007). Before planting in the experiment at Gross-Enzersdorf (irrigated trial), the soil hydraulic properties of field capacity (FC) and permanent wilting point (PWP) were determined. The average annual precipitation (1971-2000) was 520 mm. The amount of precipitation, average temperature and applied irrigation water from March to September in 2007-08 are shown in Figure 1.

Table 1. Selected properties of the experimental soil at two field trials.

	Gross-Enzersdorf (Irrigated)	Raasdorf (Rain-fed)
Texture	Silty loam	Silty loam
Organic carbon content (%)		
0-30 cm	1.5	2.0
30-60 cm	1.4	0.7
Depth of A horizon	45-50 cm	25-35 cm
Bulk density (g cm^{-1})	1.4-1.6	1.3-1.4 (Pietsch *et al.*2007)

Raasdorf (Rain-fed) 2007 Gross-Enzersdorf (Irrigated)



2008

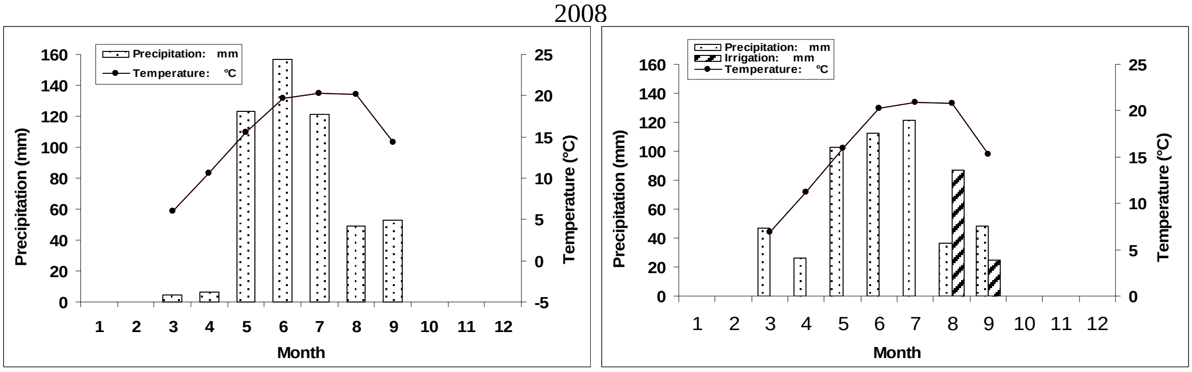


Figure 1.Monthly precipitation, average temperature and applied irrigation water from March to September 2007 and 2008.

Experimental treatment and design

Eighteen alfalfa cultivars and ecotypes (Table-2) containing eight Iranian ecotypes and ten European varieties were evaluated during 2006-08. Both trials were hand seeded in May, 2006. The first experimental year was considered as the establishment year. During the establishment, plots were hand clipped one time in September 2006. To estimate BNF, nine field plots in each experiment were hand seeded with a mixture of four grasses as reference crop (Table 2) so that the reference crop was on the first and the last plot of each incomplete block. The grass-mixture consisted of tall oat-grass (*Arrhenatherum elatius* var**.** Arone), red fescue (*Festuca rubra* var. Gondolin), cocksfoot-grass (*Dactylis glomerata* var. Amba) and perennial ryegrass (*Lolium perenne* var. Pimpernell). The seeding density was 25 kg ha^{-1} in all cases, adjusted by the germination rate of the cultivars.

Table 2. Name of tested genotypes, reference crops and their origin.

Variant	Alfalfa varieties, grass species[1]	Origin	Germination rate (%)
1	Mohajeran	Iran-West	92.0
2	Khorvande	Iran-West	92.0
3	Famenin	Iran-West	92.5
4	Ghargologh	Iran-Northwest	88.5
5	Ordobad	Iran-Northwest	94.5
6	Shorakat	Iran-Northwest	94.4
7	Ghara-aghaj	Iran-Northwest	86.5
8	Hokmabad	Iran-Northwest	87.5
9	Sitel	Netherlands	81.0
10	Verko	Hungry	97.5
11	Vlasta	Czech Republic	95.0
12	Monz42	Slovakia	85.0
13	Fix232	Slovakia	92.0
14	NS- Banat	Serbia	88.0
15	Sanditi	Netherlands	83.0
16	Alpha	Netherlands	94.0
17	Plato	Germany	85.0
18	Niva	Czech Republic	94.0
RC[1]	Arone (*A. elatius*)		54
	Gondolin (*F. rubra*)		89
	Amba (*D. glomerata*)		83
	Pimpernell (*L. perenne*)		90

RC = Reference crop; 1= Grass mixture consisting of 25% of each of species.

The field plots in both experiments, were laid out in an α-lattice design with two complete blocks or replications. Each replication consisted of three incomplete blocks and each incomplete block consisted of six experimental alfalfa plots that were surrounded by one reference crop plot at each side. All cultivars were present in each complete block or replication. Each genotype was seeded in 12 rows with 1.5-m length in the rain-fed trial at Raasdorf and 8 rows with 1-m length in the irrigated trial at Gross-Enzersdorf. Spacing between rows in both trials was 12.5 cm. In both experiments, nine plots of reference crop were seeded similar to alfalfa plots. In the irrigated trial, soil moisture content was monitored weekly by four FDR (Frequency Domain Reflectometry, ThetaProbe ML2x, UMS GmbH, München, Germany) probes in 15, 40, 80 and 120 cm soil depths; these devices were installed in one plot in each incomplete block including cultivars of 1, 9 and 18 in each replication. Irrigation was started at 50 % depletion of soil available water (SAW) content (SAW = Water content difference between field capacity and permanent wilting point) based on FDR probe in 15 cm soil depth. The amount of applied irrigation water was calculated for 0-30 cm depth based on soil moisture content up to field capacity. Plots were irrigated by a drip irrigation system. There were 28 drippers per plot with 2 litres water exit per hour and dripper. The site classification was mainly related to level of summer drought stress (irrigated and rain-fed cropping) and differing water holding capacity of locations (depth of A horizon and organic matter content of soil).

Data collection

Plots were hand clipped three times at 30-40 % of flowering using a garden scissor to a 5-cm stubble height on 1-2 June, 1-2 August and 20-21 September in the irrigated trial and 7-8 June, 11-12 August and 29-30 September in the rain-fed trial in 2007; 5-6 June, 19-20 July and 15-16 September in irrigated trial and 11-12 June, 28-29 July and 29-30 September in rain-fed trial in 2008, respectively. Root dry matter, stubble dry matter and inorganic nitrogen in 30-60 cm and 60-90 cm soil depths were recorded only at the third harvest in each year. Shoot (SHDM) and stubble (STDM) yield data (t ha^{-1}) were adjusted to a dry matter basis by sub-sampling approximately 200 and 50 g of fresh shoot and stubble, respectively, from 0.5 m^2 of the plots at each harvest, and drying the samples at 60 °C for 72 h. Annual shoot dry matter production was determined by summing the yield data over the harvests within each year. Root dry matter (RODM) (t ha^{-1}) was determined using a soil corer with 9 cm diameter. Two samples were taken in each plot down to 30 cm depth and fresh root after washing was dried at 60°C for 72 h.

Biological Nitrogen Fixation (BNF) was estimated by the “extended difference method” for each plot (Giller 2001). Based on this method, the BNF of the legume crop was taken as the difference between the total N uptake of the legume and that of the non-nodulating plant (reference crop) where both were grown at the same time on the same field regarding differences in soil inorganic N contents between the plots as well. Plant samples were taken at the harvesting time. Nitrogen content was determined in dry plant organs with an isotope ratio mass spectrometer (IRMS-ThermoQuest Finnigan DELTA plus, Bremen, Germany) in the laboratory of the Department of Chemical Ecology, University of Vienna. According to this method Biological Nitrogen Fixation Capacity (BNF [kg ha^{-1}]) is calculated with the data from the plant analyses (without regarding crop litter N) as follows:

$$\text{BNF [kg ha}^{-1}] = (\text{Shoot N}_{Leg} + \text{Stubble N}_{Leg} + \text{Root N}_{Leg}\ [\text{kg ha}^{-1}]) -$$
$$(\text{Shoot N}_{Ref} + \text{Stubble N}_{Ref} + \text{Root N}_{Ref}\ [\text{kg ha}^{-1}]) + (\text{N}_{in}\ \text{in soil}_{Leg} - \text{N}_{in}\ \text{in soil}_{Ref}\ [\text{kg ha}^{-1}])$$

which shoot N_{Leg} , stubble N_{Leg} and root N_{Leg} are nitrogen content in alfalfa shoots, stubble and roots; shoot N_{Ref} , stubble N_{Ref} and root N_{Ref} are nitrogen content in shoots, stubble and roots of reference crop (Grass mixtures); N_{in} in $soil_{Leg}$ and N_{in} in $soil_{Ref}$ are inorganic soil nitrogen contents under alfalfa and reference crop at the time of harvest, respectively.

Drought tolerance indices

Five suggested drought tolerance selection indices- tolerance (TOL), mean productivity (MP), geometric mean productivity (GMP), stress susceptibility index (SSI) and stress tolerance index (STI) along with superiority statistic (P_i)- were calculated using the Excel spread sheet program as follows:

$$\text{Tol} = Y_p - Y_s \quad \text{(Rosielle and Hamblin 1981)}$$

$$\text{MP} = \frac{Y_p + Y_S}{2} \quad \text{(Rosielle and Hamblin 1981)}$$

$$\text{SSI} = \frac{1 - \frac{Y_s}{Y_p}}{\text{SI}}, \ \text{SI (Stress intensity)} = 1 - \frac{\overline{Y}_s}{\overline{Y}_p} \quad \text{(Fischer and Maurer 1987)}$$

$$\text{GMP} = \sqrt{Y_s \times Y_p} \quad \text{(Fernandez 1992)}$$

$$\text{STI} = \frac{Ys \times YP}{\overline{Y}_p^2} \quad \text{(Fernandez 1992)}$$

$$P_i = \frac{\sum_{j=1}^{q} (X_{ij} - M_j)^2}{2q} \quad \text{(Lin and Binns 1988)}$$

Where Ys and Yp are the yield of genotype in stress and non-stress conditions, $\overline{Y}_s$ and $\overline{Y}_p$ are the mean yield over all genotypes evaluated under stress and non-stress conditions, X_{ij} is the yield of genotype i in environment j , M_j the yield of the best genotype in environment j and q number of environment,. Stress intensity (SI) ranges between 0 and 1, and larger values of SI indicate higher stress intensity. Larger values of MP, GMP and STI and the smaller values of Pi, TOL and SSI are desired while for all indices a low rank is desired.

Statistical analysis

The data were analyzed based on repeated measure analysis of variance based on an alpha-lattice design. A linear mixed model was used, where location (L), replication (Rep) and genotype (G) were considered as fixed effects, while incomplete block within replication [iblock (rep)] and year (Y) were considered as random effects and repeated measure, respectively. The analysis was done using two different covariance structures, the unstructured (UN) and the first-order autoregressive AR (1). The Akaike Information Criterion (AIC) was used to find the best model describing the covariance structure. The data were analyzed by PROC MIXED in SAS software (SAS Institute, 2004). Denominator degrees of freedom (DDF) were calculated using the Kenward-Roger (KR) method. Adjusted least square (LS) means were estimated for each condition, and over two conditions were used to calculation different indices. Mean comparisons were adjusted for the *p*-values (α =0.05) using ADJUST=SIMULATION option in SAS software. Regarding genotype by location interaction (GL), LS-mean comparisons have been carried out among cultivars in each location. A SAS macro was used to find a letters display for all pairwise mean comparisons (Piepho, 2009). Simple correlations were calculated based on genotype means between drought tolerance indices, rain-fed and irrigated perfoemances of genotypes (n=18) using SPSS software (version 15).

Results

The results of analysis of variance and mean comparisons were cited in chapter 1. The mean values of genotypes for shoot dry matter (SHDM), total biomass yield (TBY) and BNF under rain-fed (RF) and irrigated (IR) conditions in the first, second and average over two years are given in Table 3. The stress intensities (SI) in the first year for SHDM, TBY and BNF were 0.48, 0.38 and 0.35 and in second year 0.25, 0.20 and 0.14, respectively (Table 4). Thus, the first year, the average over two years and the second year of study were considered as high, medium and low stress pressure, respectively. To identify the best stress tolerance index to

Table 3. Shoot DM, total biomass and BNF yield of genotypes under irrigated and rain-fed conditions during the first, the second and on the average over two years.

	Shoot DM (t ha^{-1})						Total biomass (t ha^{-1})						BNF (kg ha^{-1})					
	Irrigated			Rain-fed			Irrigated			Rain-fed			Irrigated			Rain-fed		
Genotype	1st	2nd	Ave.	1st	2nd	Ave.	1st	2nd	Ave.	1st	2nd	Ave.	1st	2nd	Ave.	1st	2nd	Ave.
Mohajeran	19.6	17.3	18.4	7.8	11.0	9.4	28.9	26.8	27.8	14.3	21.3	17.8	491	427	459	260	336	298
Khorvande	10.6	12.2	11.4	6.2	9.4	7.8	20.9	25.6	23.3	12.0	19.3	15.7	292	381	336	193	306	249
Famenin	14.4	16.5	15.5	9.7	10.9	10.3	23.1	26.9	25.0	15.5	20.2	17.8	327	372	350	253	294	274
Gharghologh	12.6	14.7	13.6	7.0	12.6	9.8	24.3	26.9	25.6	16.0	22.6	19.3	381	393	387	263	358	310
Ordobad	15.7	14.6	15.1	5.5	11.0	8.3	27.0	25.2	26.1	12.2	20.3	16.2	411	380	395	155	353	254
Shorakat	17.6	15.1	16.3	6.4	13.3	9.8	28.8	23.6	26.2	16.1	23.6	19.9	430	378	404	266	401	334
Ghara-aghaj	13.9	17.2	15.5	9.8	13.2	11.5	22.3	25.3	23.8	17.8	22.0	19.9	304	375	340	304	364	334
Hokmabad	14.4	14.3	14.3	6.3	10.5	8.4	24.6	25.3	25.0	14.9	19.4	17.1	407	402	405	234	271	252
Sitel	17.1	17.7	17.4	10.9	13.7	12.3	24.0	30.1	27.1	16.6	23.6	20.1	411	459	435	273	413	343
Verko	17.2	17.6	17.4	7.5	12.0	9.7	27.3	26.2	26.7	12.9	20.9	16.9	478	418	448	183	388	286
Vlasta	15.8	17.0	16.4	10.8	12.6	11.7	23.8	25.8	24.8	21.8	22.1	21.9	386	424	405	381	390	385
Monz 42	15.5	15.5	15.5	8.0	10.1	9.1	23.1	26.9	25.0	15.7	18.3	17.0	367	399	383	249	265	257
Fix 232	17.5	17.0	17.2	7.9	13.3	10.6	28.4	30.0	29.2	13.1	21.4	17.2	487	505	496	209	394	301
NS-Banat	16.2	16.0	16.1	7.7	14.0	10.9	28.5	28.2	28.4	14.9	22.4	18.6	479	491	485	275	383	329
Sanditi	14.7	15.8	15.3	10.6	12.7	11.6	21.0	24.9	23.0	17.4	21.8	19.6	335	346	341	328	343	336
Alpha	16.0	17.6	16.8	7.4	11.5	9.5	21.5	26.6	24.0	12.5	20.5	16.5	318	387	353	188	379	283
Plato ZS	17.6	16.4	17.0	9.0	12.7	10.9	25.1	25.4	25.3	17.3	20.6	18.9	461	423	442	318	365	341
Niva	15.0	18.1	16.5	8.5	13.7	11.1	20.4	29.6	25.0	14.6	23.0	18.8	333	490	411	252	398	325
Mean	15.6	16.1	15.9	8.2	12.1	10.1	24.6	26.6	25.6	15.3	21.3	18.3	394	414	404	255	356	305

1st, 2nd and Ave. are first, second and average of two years of study, respectively.

Table 4. Stress intensity (SI) calculated for shoot DM, total biomass yield and BNF in the first, the second and on the average over two years of study.

Trait	1st year	2nd year	Average
Shoot DM	0.48	0.25	0.36
Total biomass yield	0.38	0.20	0.29
BNF	0.35	0.14	0.24

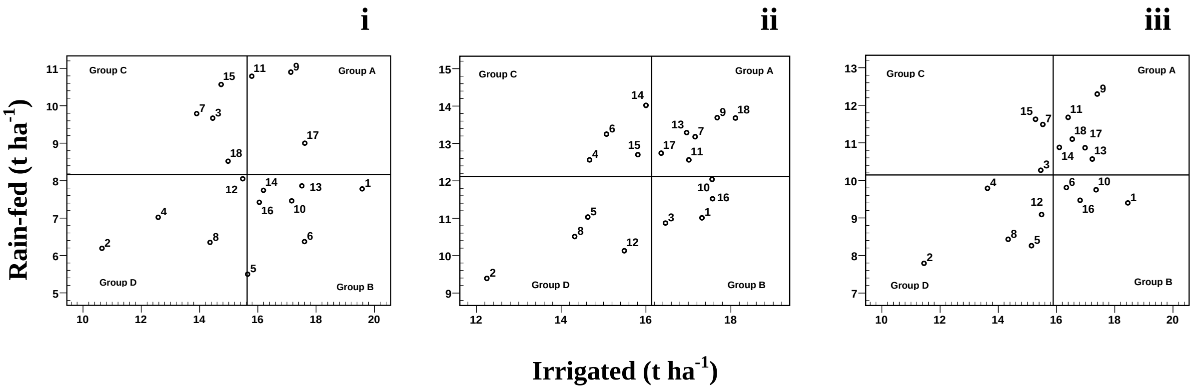


Fig. 2. Scatter plot of shoot DM yield (t ha^{-1}) of genotypes under rain-fed and irrigated conditions during the first (i), the second (ii) and on the average over two years (iii).

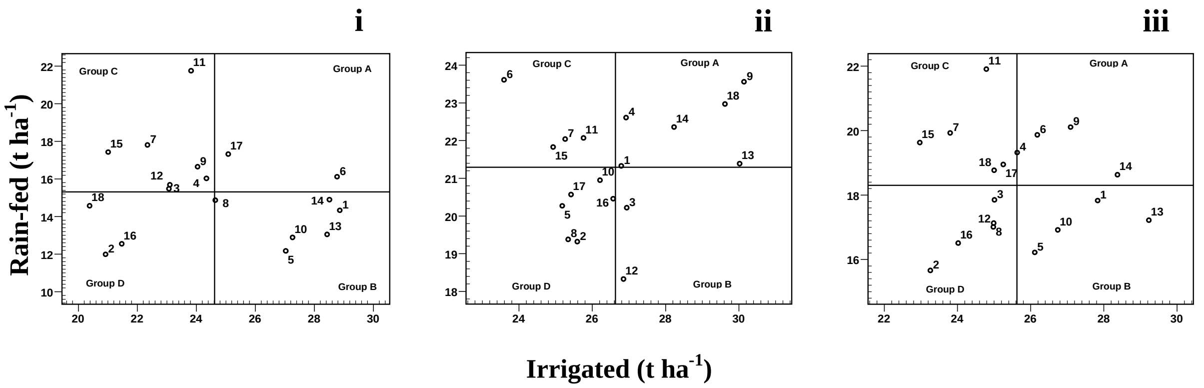


Fig. 3. Scatter plot of total biomass yield (t ha^{-1}) of genotypes under rain-fed and irrigated conditions in the first (i) and the second year (ii) and on the average over two years (iii).

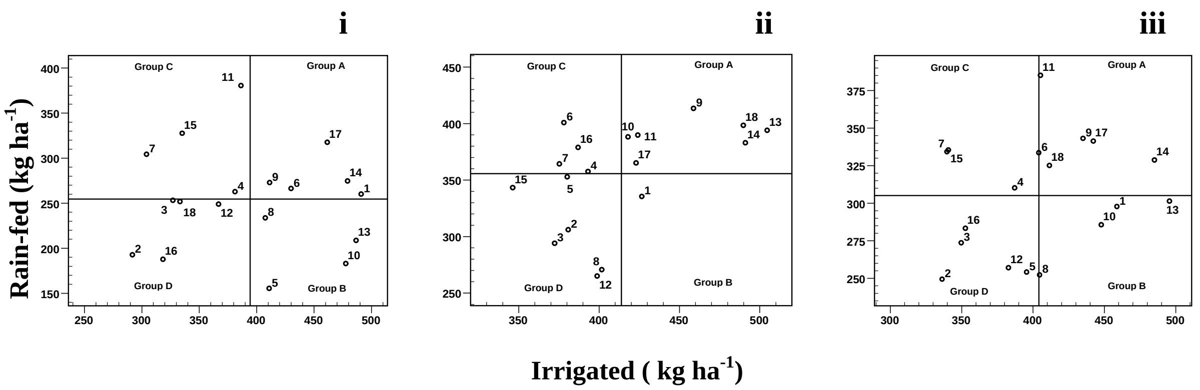


Fig. 4. Scatter plot of BNF estimated (kg ha^{-1}) of genotypes under rain-fed and irrigated conditions in the first (i) and the second (ii) year and on the average over two years (iii).

distinguish superior genotypes for both conditions, the genotypes were categorized into four groups (A to D) (Fernandez 1992) in scatter plots by drawing reference lines through x (irrigated) and y (rain-fed) axes using the grand mean of all genotypes in each condition (Figs. 2 to 4). For all three characters, the number of selected superior genotypes using different indices was derived from the number of genotypes classified in Group A (Figs. 2 to 4) for each condition.

Shoot dry matter (SHDM)

The highest yield under the IR condition was shown by ecotype Mohajeran in the first year and on the average over two years, and by Niva in the second year. Under the RF condition, Sitel showed the highest yield in the first and on the average over two years, and NS-Banat in the second year (Table 3). The correlation coefficients between SHDM under IR and RN conditions for high (first year), medium (average over two years) and low (second year) SI were 0.13, 0.49* and 0.56*, respectively (Table 8). In high, medium and low SI, 3, 6 and 6 genotypes were identified as Group A, respectively. The genotypes 9 (Sitel), 17 (Plato) and 11 (Vlasta) in first year (high SI) (Fig 2i); the genotypes 18 (Niva), 9 (Sitel), 7 (Ghara-aghaj), 13 (Fix232), 11 (Vlasta) and 17 (Plato) in second year (low SI) (Fig. 2ii); and the genotypes 9 (Sitel), 11 (Vlasta), 13 (Fix232), 17 (Plato), 18 (Niva) and 14 (NS-Banat) based on average over two years (medium SI) (Fig. 2iii) were categorized in Group A.The rank of genotypes based on different indices is presented in Table 5.

Table 5. Ranks of genotypes for different stress tolerance indices using shoot DM yield.

Index	TOL			MP			GMP			SSI			STI			Pi		
Genotype	1^{st}	2^{nd}	Ave.	1^{st}	2^{nd}	Ave.	1^{st}	2^{nd}	Ave.	1^{st}	2^{nd}	Ave.	1^{st}	2^{nd}	Ave.	1^{st}	2^{nd}	Ave.
Mohajeran	18	18	18	2	11	4	5	12	9	16	18	18	5	12	9	3	11	5
Khorvande	3	4	2	18	18	18	18	18	18	6	9	6	18	18	18	18	18	18
Famenin	4	16	7	8	13	13	6	14	13	4	15	9	6	14	13	13	13	13
Ghargologh	6	3	3	17	14	15	16	13	15	8	3	3	16	13	15	17	14	17
Ordobad	16	6	15	15	15	16	17	15	16	18	11	17	17	15	16	15	15	15
Shorakat	17	1	13	9	12	12	14	11	11	17	1	12	14	11	11	9	12	11
Ghara-aghaj	1	10	4	11	3	8	8	3	6	2	8	2	8	3	6	14	3	12
Hokmabad	10	9	10	16	17	17	15	17	17	14	13	13	15	17	17	16	17	16
Sitel	7	11	6	1	2	1	1	2	1	5	7	5	1	2	1	1	2	1
Verko	15	15	17	7	6	7	9	7	10	15	14	16	9	7	10	6	6	6
Vlasta	5	13	5	4	7	2	2	6	2	3	12	4	2	6	2	5	5	4
Monz 42	9	14	12	12	16	14	12	16	14	9	17	14	12	16	14	11	16	14
Fix 232	14	8	14	5	4	5	7	4	5	13	5	11	7	4	5	4	4	3
NS-Banat	11	2	8	10	5	9	11	5	8	11	2	7	11	5	8	7	7	7
Sanditi	2	5	1	6	10	10	4	10	7	1	4	1	4	10	7	8	10	9
Alpha	13	17	16	14	9	11	13	9	12	12	16	15	13	9	12	10	9	10
Plato ZS	12	7	11	3	8	3	3	8	3	10	6	10	3	8	3	2	8	2
Niva	8	12	9	13	1	6	10	1	4	7	10	8	10	1	4	12	1	8

See Table 3 for abbreviations.

Based on TOL, none of the Group A genotypes was selected in high and low SI, whereas in medium SI, only Sitel and Vlasta were identified. Based on MP, most of the Group A genotypes- Sitel and Plato ZS in first year (high SI), Niva, Sitel, Ghara-aghaj, Fix232 and NS-

Banat in second year (low SI), and Sitel, Vlasta, Plato ZS, Fix232 and Niva on average over two years (medium SI)- were differentiated from other groups. The correlations between MP and SHDM were positive and significant under both IR and RF conditions (Table 8). Based on SSI, only Vlasta in high SI, Fix232 and Plato ZS in low SI, and Sitel and Vlasta in medium SI were distinguished as Group A genotypes. There were positive correlations between SSI and SHDM under IR conditions, while they were significant and negative under RF conditions in high, medium and low SI (Table 8).

The results of GMP and STI were identical in ranking. Based on STI, all Group A genotypes were distinguished from other groups, except for Plato ZS (17) in low SI and NS-Banat (14) in medium SI (Table 5). In high SI, STI was able to distinguish all Group A genotypes. In medium SI, NS-Banat (14), as a Group A genotype, was ranked after Ghara-aghaj (7) and Sanditi (15) from Group C with high rain-fed yields, and in low SI, Plato ZS (17) was ranked after NS-Banat (14) from Group C and Verko (10) from Group B. The correlations between STI and SHDM in both conditions were positive and significant (Table 8). However, the association of STI with SHDM under RF condition was greater than of that under IR condition.Using Pi, the most of the Group A genotypes were discriminated, except for Vlasta (11) in high SI, Plato ZS (17) in low SI, and NS-Banat (14) and Niva (18) in medium SI.There were negative and significant correlations between Pi and SHDM (Table 8), showing that selecting by Pi will increase yield in both conditions.

Total biomass yield (TBY)

Mohajeran, Sitel and Fix232 under IR conditions and Vlasta, Shorakat and Vlasta under RF conditions had the greatest total biomass yield in the first, the second and on the average over two years, respectively (Table 3). There were non-significant correlations in high ($r = -0.17$), medium ($r = -0.04$) and low SI ($r = 0.26$) between TBY of genotypes under IR and RF conditions (Table 9). Two, six and four genotypes were selected as Group A in high, low and medium SI, respectively (Fig. 3).

The rank of genotypes for different indices is presented in Table 6. In high SI, TOL and SSI were not able to identify Group A genotypes, while other indices distinguished only Shorakat from Group A (Table 6 and Fig. 3i). In low SI, TOL and SSI only distinguished one genotype (Gharghologh (4)) out of six genotypes in Group A, while other indices identified all Group A genotypes (Table 6 and Fig. 3i). Fix 232, as a member of Group A genotypes, was ranked as an unsuitable genotype by TOL and SSI because of the large yield difference between the two conditions (Tables 3 and 6). In medium SI, TOL could not distinguish Group A genotypes, while GMP, STI and Pi identified the most of these genotypes (Table 6 and Fig.3iii). All indices ranked Vlasta as first because of the high total biomass yield under rain-fed condition in high and medium SI. The correlations between indices and TBY in IR and RF condition were similar to SHDM (Table 9).

Table 6. Ranks of genotypes for different stress tolerance indices using total biomass yield.

Index	TOL			MP			GMP			SSI			STI			Pi		
Genotype	1^{st}	2^{nd}	Ave.	1^{st}	2^{nd}	Ave.	1^{st}	2^{nd}	Ave.	1^{st}	2^{nd}	Ave.	1^{st}	2^{nd}	Ave.	1^{st}	2^{nd}	Ave.
Mohajeran	16	9	17	4	6	6	5	6	6	15	9	15	5	6	6	7	6	6
Khorvande	11	13	11	18	17	18	18	16	18	12	15	13	18	16	18	18	16	18
Famenin	7	16	9	14	11	12	13	11	13	8	16	9	13	11	13	11	8	10
Ghargholoch	9	5	6	8	5	7	8	5	7	9	5	5	8	5	7	6	5	4
Ordobad	17	7	16	12	15	14	15	15	16	18	7	17	15	15	16	15	14	16
Shorakat	13	1	7	2	9	5	2	8	4	13	1	4	2	8	4	2	18	7
Ghara-aghaj	3	3	3	10	8	10	7	9	9	3	3	3	7	9	9	8	11	9
Hokmabad	12	11	12	11	18	15	10	18	14	10	14	11	10	18	14	9	17	12
Sitel	5	14	8	7	1	1	6	1	1	5	11	8	6	1	1	5	1	2
Verko	15	8	15	9	10	11	14	10	11	16	8	16	14	10	11	14	9	11
Vlasta	1	4	1	1	7	3	1	7	2	1	4	1	1	7	2	1	7	1
Monz 42	6	17	13	13	16	16	12	17	15	7	18	12	12	17	15	10	15	13
Fix 232	18	18	18	6	3	4	9	3	5	17	17	18	9	3	5	12	3	8
NS-Banat	14	10	14	3	4	2	4	4	3	14	10	14	4	4	3	4	4	3
Sanditi	2	2	2	15	13	13	11	12	12	2	2	2	11	12	12	13	12	14
Alpha	10	12	10	17	12	17	17	13	17	11	13	10	17	13	17	17	10	17
Plato ZS	8	6	5	5	14	8	3	14	8	6	6	7	3	14	8	3	13	5
Niva	4	15	4	16	2	9	16	2	10	4	12	6	16	2	10	16	2	15

See Table 3 for abbreviations.

Table 7. Ranks of genotypes for different stress tolerance indices using BNF.

Index	TOL			MP			GMP			SSI			STI			Pi		
Genotype	1^{st}	2^{nd}	Ave.	1^{st}	2^{nd}	Ave.	1^{st}	2^{nd}	Ave.	1^{st}	2^{nd}	Ave.	1^{st}	2^{nd}	Ave.	1^{st}	2^{nd}	Ave.
Mohajeran	15	13	16	4	10	6	4	10	6	15	14	14	4	10	6	4	8	5
Khorvande	6	11	9	18	15	18	18	15	18	10	12	11	18	15	18	18	14	18
Famenin	4	12	6	15	17	17	15	16	17	4	13	9	15	16	17	15	17	15
Ghargholoch	8	8	7	10	11	10	9	11	10	6	8	6	9	11	10	7	10	8
Ordobad	16	5	13	16	13	14	16	13	14	18	6	15	16	13	14	16	13	16
Shorakat	12	1	5	5	8	7	5	8	7	11	1	4	5	8	7	5	11	7
Ghara-aghaj	1	4	2	13	12	12	11	12	12	1	4	2	11	12	12	13	12	13
Hokmabad	13	17	14	11	16	13	10	17	13	13	17	17	10	17	13	9	16	12
Sitel	10	9	10	7	4	5	6	3	5	9	9	8	6	3	5	6	4	4
Verko	18	6	17	9	6	9	13	6	9	17	5	16	13	6	9	12	6	10
Vlasta	2	7	3	2	5	3	1	5	2	2	7	3	1	5	2	2	5	3
Monz 42	7	18	12	12	18	15	12	18	16	8	18	13	12	18	16	11	18	14
Fix 232	17	16	18	6	1	2	8	1	4	16	15	18	8	1	4	10	1	6
NS-Banat	14	15	15	3	3	1	3	4	1	14	16	12	3	4	1	3	3	1
Sanditi	3	2	1	8	14	11	7	14	11	3	2	1	7	14	11	8	15	11
Alpha	9	3	4	17	9	16	17	9	15	12	3	5	17	9	15	17	9	17
Plato ZS	11	10	11	1	7	4	2	7	3	7	10	10	2	7	3	1	7	2
Niva	5	14	8	14	2	8	14	2	8	5	11	7	14	2	8	14	2	9

See Table 3 for abbreviations.

Table 8. Simple correlation between drought tolerance indices, irrigated and rain-fed shoot DM.

		SHDM Irrigated	SHDM Rain-fed	TOL	MP	GMP	SSI	STI
SHDM Rain-fed	1st	0.13						
	2nd	0.56*						
	Ave.	0.49*						
Tol	1st	0.75**	-0.56*					
	2nd	0.55*	-0.39					
	Ave.	0.66**	-0.33					
MP	1st	0.81**	0.68**	0.23				
	2nd	0.89**	0.87**	0.12				
	Ave.	0.90**	0.83**	0.26				
GMP	1st	0.62**	0.86**	-0.05	0.96**			
	2nd	0.86**	0.90**	0.04	1**			
	Ave.	0.83**	0.89**	0.13	0.99**			
SSI	1st	0.46	-0.81**	0.93**	-0.13	-0.40		
	2nd	0.32	-0.61**	0.96**	-0.14	-0.22		
	Ave.	0.36	-0.63**	0.93**	-0.09	-0.22		
STI	1st	0.59*	0.87**	-0.09	0.95**	1**	-0.44	
	2nd	0.85**	0.91**	0.03	0.99**	1**	-0.23	
	Ave.	0.82**	0.90**	0.11	0.99**	1**	-0.24	
PI	1st	-0.86**	-0.54*	-0.36	-0.95**	-0.87**	-0.03	-0.84**
	2nd	-0.88**	-0.81**	-0.16	-0.96**	-0.95**	0.09	-0.93**
	Ave.	-0.92**	-0.72**	-0.39	-0.96**	-0.94**	-0.06	-0.92**

SHDM: shoot dry matter; see Table 3 for abbreviations. * and ** are significant at 5 and 1 % probability level, resp.

Biological nitrogen fixation (BNF)

With regard to BNF, the highest estimations were as follows: under IR condition – Mohajeran in the first year and Fix232 in the second and on the average over two years; under RF condition – Vlasta in the first and on the average over two years and Sitel in the second year of study (Table 3). The correlations between BNF of genotypes under IR and RF conditions were -0.05, 0.22 and 0.48* in high, medium and low SI, respectively (Table 10). Five, seven and six genotypes were classified as Group A in high, low and medium SI, respectively (Fig. 4). The rank of genotypes for different indices is presented in Table 7.

Similar to SHDM and TBY, TOL and SSI were not able to identify Group A genotypes in high SI; only Verko and Vlasta in low SI and Vlasta and Shorakat in medium SI were distinguished from the other groups. With minor changes in ranking, MP, STI and Pi had the same efficiency in separating Group A genotypes from the other groups (Table 7 and Fig. 4). They were able to identify most of the Group A genotypes as being separate from the other groups.

Table 9. Simple correlation between drought tolerance indices, irrigated and rain-fed total biomass yield.

		TBY Irrigated	TBY Rain-fed	TOL	MP	GMP	SSI	STI
TBY Rain-fed	1st	-0.17						
	2nd	0.26						
	Ave.	-0.04						
Tol	1st	0.81**	-0.72**					
	2nd	0.71**	-0.50*					
	Ave.	0.74**	-0.71**					
MP	1st	0.72**	0.56*	0.18				
	2nd	0.84**	0.74**	0.21				
	Ave.	0.71**	0.67**	0.04				
GMP	1st	0.51*	0.76**	-0.10	0.96**			
	2nd	0.79**	0.79**	0.14	1**			
	Ave.	0.58*	0.79**	-0.12	0.98**			
SSI	1st	0.65**	-0.85**	0.97**	-0.05	-0.31		
	2nd	0.59*	-0.63**	0.98**	0.05	-0.02		
	Ave.	0.60**	-0.83**	0.98**	-0.14	-0.30		
STI	1st	0.48*	0.78**	-0.12	0.95**	1**	-0.34	
	2nd	0.80**	0.79**	0.14	1**	1**	-0.02	
	Ave.	0.58*	0.78**	-0.12	0.99**	1**	-0.30	
PI	1st	-0.53*	-0.70**	0.04	-0.93**	-0.97**	0.25	-0.95**
	2nd	-0.87**	-0.58*	-0.36	-0.93**	-0.92**	-0.24	-0.92**
	Ave.	-0.54*	-0.73**	0.12	-0.92**	-0.93**	0.28	-0.93**

TBY: total biomass yield; see Table 3 for abbreviations. * and ** are significant at 5 and 1 % probability level, resp.

Table 10. Simple correlation between drought tolerance indices, irrigated and rain-fed BNF.

		BNF Irrigated	BNF Rain-fed	TOL	MP	GMP	SSI	STI
BNF Rain-fed	1st	-0.05						
	2nd	0.48*						
	Ave.	0.22						
Tol	1st	0.78**	-0.67**					
	2nd	0.52*	-0.51*					
	Ave.	0.73**	-0.50*					
MP	1st	0.75**	0.63**	0.16				
	2nd	0.86**	0.86**	0.01				
	Ave.	0.84**	0.72**	0.24				
GMP	1st	0.57*	0.79**	-0.07	0.97**			
	2nd	0.84**	0.88**	-0.04	1**			
	Ave.	0.78**	0.79**	0.14	0.99**			
SSI	1st	0.62**	-0.80**	0.97**	-0.05	-0.27		
	2nd	0.38	-0.63**	0.99**	-0.14	-0.19		
	Ave.	0.59*	-0.65**	0.98**	0.05	-0.05		
STI	1st	0.56*	0.79**	-0.08	0.96**	1**	-0.28	
	2nd	0.85**	0.86**	0.00	1**	1**	-0.16	
	Ave.	0.77**	0.79**	0.14	0.99**	1**	-0.05	
PI	1st	-0.63**	-0.70**	-0.03	-0.96**	-0.97**	0.17	-0.96**
	2nd	-0.82**	-0.86**	0.04	-0.98**	-0.98**	0.18	-0.98**
	Ave.	-0.77**	-0.75**	-0.16	-0.96**	-0.97**	0.02	-0.97**

BNF: biological nitrogen fixation; see Table 3 for abbreviations. * and ** are significant at 5 and 1 % probability level, resp.

The correlation between TOL and SSI was positive, large and significant for all three studied characters. MP, GMP, STI and Pi were highly correlated to each other (Tables 8 to 10). There were no significant correlations between TOL or SSI and other indices in the three characters. In average over these characters, STI, GMP, MP, Pi, SSI and TOL differentiated about 77, 77, 66, 66, 11 and 0 % of genotypes in Group A in high SI; 75, 75, 67, 69, 30 and 22 % in medium SI; and 94, 94, 89, 94, 26 and 15 % in low SI, respectively.

Discussion

Year-to-year fluctuations occurred in the amount, frequency, time and duration of rainfall under rain-fed condition. Although the amount of precipitation did not differ considerably, mid-term and late season stress was imposed on genotypes during the first and second year of the study, respectively (Fig. 1). The SI values (Table 4) showed that, in addition to different stress intensity during the two years of the experiment due to rainfall fluctuations, the drought stress had different effects on different plant parts. SHDM was reduced the most (compared with TBY and BNF) in both years due to drought conditions. This parallels the results of Showemimo and Olarewaju (2007), who reported different effects of drought stress on various traits of sweet pepper (*Capsicum annuum* L.).

The relationship between the performance of genotypes under IR and RF conditions (Tables 8 to 10) were affected by the level of SI and by the traits. The strength of the relation between the two conditions increased with decreasing stress intensity (SI) from the first to second year of the study, suggesting that selection of the drought-tolerant genotypes based on the yield in non-stress conditions is unreliable, especially under high SI. Moreover, the degree of association between the two conditions differed among the studied traits. SHDM, as a constituent of TBY, showed a stronger relation between the performances under the two conditions than TBY and BNF. This indicates the role of non-harvestable plant parts, especially root DM, in the selection process for tolerant or Group A genotypes. On the other hand, the degree of association between the two conditions influenced the number of genotypes in Group A. The number of genotypes in Group A decreased with increasing SI (Figs 2 to 4). This shows the weak positive and occasionally negative correlations between IR and RF conditions (Tables 9 and 10). Also, we observed the same trend of fewer Group A genotypes for TBY and BNF compared to SHDM. This reduction suggests a reduced possibility of finding genotypes that express uniform superiority in both stress and non-stress conditions.

Considering the variable relation between performances under stress and non-stress conditions, the simultaneous assessment of genotypes under both conditions (third approach mentioned in Introduction) can be a confident way to identify suitable genotypes for rain-based cropping systems.

With regard to correlation between IR or RF performance and drought tolerance indices, genotype selection based on TOL and SSI will increase overall yield only under the RF condition, while MP, GMP, STI and Pi will increase it under both conditions. In the current study, MP and STI (or GMP) were the best mean predictors of IR and RF performances, respectively. Akhtar *et al*., (2000) reported that MP would best identify genotypes with high performance in non-stress environments, and GMP would best identify genotypes with high performance in stress environments. Sio-Se Mardeh *et al.*, (2006) found a negative and significant correlation between Pi and yield of wheat genotypes under non-stress condition, whereas it was positive and non-significant under stress condition.

Fernandez (1992), however stated that identifying Group A genotypes is a better approach than correlation analysis because the effectiveness of genetic gain based on the observed correlation may not reflect the genetic gain of individual genotypes. TOL and SSI were unsuitable indices to identify Group A genotypes, although some genotypes like Vlasta were selected by them due to high yield under rain-fed conditions. Under stress conditions, SSI can be applied to improve yield in future alfalfa studies with regard to the high association between TOL and SSI (Tables 8 to 10) and because SSI is more informative than TOL. In general, SSI was a suitable index to identify genotypes, mostly those in Group C, with high performance under stress conditions, such as Sanditi, Vlasta and Ghara-aghaj. The correlation between SSI and rain-fed performance was stronger in high SI than medium and low SI, confirming the suitability of SSI to select tolerant and high-yielding genotypes in stress conditions.

Although most of Group A genotypes were distinguished by MP, GMP, STI and Pi (Tables 5 to 7 and Figs 2 to 4), STI and GMP were the best indices to separate the Group A genotypes with regard to correlations among these criteria (Tables 8 to 10) and based on better efficiency in ranking of genotypes. Farshadfar and Sutka (2003) suggested MP, GMP and STI as the most suitable drought-tolerance criteria for screening wheat substitution lines. Fernandez (1992) and Akhtar *et al.*, (2000) concluded that STI is the best index to separate Group A genotypes in mungbean *(Vigna radiata* L. Wilczek). However, the suitability of indices decreased with increasing SI (regarding the number of identified Group A genotypes), showing that their ability depends on the severity of stress (Tables 5 to 7 and Figs 2 to 4).

In conclusion, the utility of different approaches for a breeding strategy for stress environments depends on stress intensity. In low SI, when the correlation between yield under stress and non-stress conditions is moderate to high and yield reduction due to stress condition is low, the first approach (selection under good conditions and subsequent yield testing in stress environments) apparently works better than others. In high SI, when the correlation between the yield under stress and non-stress conditions is low and sometimes negative and yield losses due to stress condition is high, the second approach (direct selection in target stress conditions) apparently works better than others. Based on the present and other studies, however, the third approach – simultaneous evaluation of genotypes under both conditions – ensures the selection of superior genotypes for both conditions, especially in moderate stress intensity.

Although this study was conducted under organic conditions, it may be inferred that, if yield reduction in organic versus conventional farming is high and significant, then the specific breeding program should be planned to develop varieties for organic conditions as the target environment. Annicchiarico *et al*. (2006) reported that cultivar by environment interaction effects and their implications on selection and recommendation of alfalfa varieties for Italian environmental conditions are more affected by the geographical area and the drought stress level than by cropping in both conventional or organic farming systems. In the present study, Sitel, Plato ZS, Vlasta and NS-Banat were the best genotypes based on their performances under both conditions. As specific adaptations, Vlasta and Sitel can be selected for stress and non-stress conditions, respectively. Among Iranian ecotypes, the best performance was achieved by Mohajeran under irrigated and Ghara-aghaj and Ghargholoh under rain-fed conditions. STI and GMP were the best indices to distinguish Group A genotypes from other groups, while SSI was the best one to separate genotypes with high yield potentials under stress conditions.

Chapter Three

Variation of Water-Use Efficiency and Carbon Isotope Discrimination among 18 Alfalfa Genotypes under Irrigated and Rain-fed Organic Farming

Variation of Water-use Efficiency and Carbon Isotope Discrimination among 18 Alfalfa Genotypes under Irrigated and Rain-fed Organic Farming

Abstract

Alfalfa is grown under both rain-fed and irrigated condition throughout the world and is usually grown as a rain-fed crop in crop rotations in organic systems in eastern Austria. Water-use efficiency (WUE) as important indicator of plant adaptation to drought is a main target of many breeding programs. Carbon isotope discrimination (Δ) has been proposed as a method and technique for evaluating and improving water use efficiency in C_3 plants. In total, 18 alfalfa genotypes from different geographical origins, 8 Iranian ecotypes and 10 European cultivars, were evaluated under irrigated and rain-fed conditions at the research station of the University of Natural Resources and Applied Life Sciences (BOKU), Vienna, Austria, during 2007-08. The objectives of this study were (i) to estimate WUE and Δ in different alfalfa genotypes, (ii) to determine the effect of drought stress on WUE and Δ, (iii) to study relationships between biomass productions, water use efficiency and carbon isotope discrimination, and (iv) to examine variation and consistency of Δ in harvestable (shoot) and non-harvestable (stubble and root) parts of produced biomass under irrigated and rain-fed conditions. Significant differences between locations (L) and years (Y) occurred for all characters. Genotype (G) by location and genotype by year interactions were significant only for water use efficiency. significant differences were found among different harvests for Δ-shoot under both conditions while genotype by harvest interaction was only significant under irrigated condition. The genotypes differed in Δ-shoot depending on harvest time and conditions, indicating inconsistent differences in Δ-shoot. The rank correlations between different harvests for Δ-shoot were low and mostly insignificant under both conditions. Sitel was the most water use efficient genotype across the two conditions followed by Mohajeran, Fix232 and Verko under irrigated condition and Vlasta, Sanditi, Ghara-aghaj under rain-fed conditions. Drought stress under rain-fed condition reduced the overall mean of water use efficiency and carbon isotope discrimination responses, but the ratios of reduction differed for characters and genotypes. Narrow ranges were found for all traits especially for WUE-TBY (total biomass yield) (0.78 kg m^{-3}) and Δ-shoot (0.53 ‰) based on genotype means over locations and years, although variation and ranges were higher under irrigated condition. The shoot was the best and additionally simplest and most economical single criterion to characterize alfalfa genotypes for high WUE via Δ in this study.

The rank correlation between two location or years was positive and mostly non significant for Δ in all plant parts in spite of non significant L × G, Y × G and L ×Y × G interaction. The relation between SHDM (shoot dry matter), TBY and consequently water use efficiency (WUE-SHDM and WUE-TBY) and Δ responses of genotypes (shoot, stubble and root) were variable based on plant part and study conditions (irrigated and rain-fed). Correlations between Δ-shoot and shoot dry matter, total biomass yield and their relevant water use efficiency were positive under irrigated condition, while they were negative under rain-fed condition. Regarding the variable and low correlations, simultaneous assessment of genotypes for Δ-shoot and biomass production can ensure the selection of superior genotypes and minimize potential biomass reductions that may result from using Δ-shoot as the only selection criterion to improve WUE.

Key words: Lucerne, shoot dry matter, total biomass yield, correlation, water stress, Iranian ecotypes.

Introduction

Alfalfa (*Medicago sativa* L.) is the world's most important forage crop (Barnes *et al.* 1988) which provides high-quality forage, fixes atmospheric nitrogen and survives in dry, high temperature environment (Johnson and Rumbaugh 1995). Legume fodder crops such as alfalfa (*Medicago sativa* L.) are an essential component of organic farming system especially in arid and semiarid conditions. Stockless organic farming is predominant in the dry, pannonian region of eastern Austria and alfalfa is the best known fodder crop with high ability of biological nitrogen fixation (BNF) and drought tolerance in this region. Alfalfa is grown under both rain-fed and irrigated conditions throughout the world and is usually grown as a rain-fed crop in crop rotations in organic systems in eastern Austria, where year-to-year fluctuations occur in the amount and particularly frequency, and duration of rainfall.

One of the most important physiological characters is water use efficiency (WUE) indicating plant adaptation to drought (Martin *et al*., 1999; Ray *et al*., 1999b). Due to steadily increasing demands for water supply and global climate changes, breeding programs for water use efficiency improvement in all crops will play an important and vital role in the future. Any improvement in water use efficiency can influence alfalfa forage production under both irrigated and rain-fed conditions. Alfalfa water use is considered relatively extravagant because of its large seasonal evapotranspiration compared to other crops (Sheaffer *et al.* 1988). Variability in water use efficiency among and within alfalfa cultivars and populations has been reported by many researchers (e.g. Cole *et al*. 1970; Johnson and Tieszen 1994; Ray *et al*. 1998). However, it is difficult to assess this trait in a field-grown plant. Because of a lack of practical techniques for screening large breeding materials for it, improvement of water use efficiency is difficult and time-consuming. Melton *et al.* (1989) stated that successful alfalfa improvement for water use efficiency requires multiple years of field assessments of germplasms.

The introduction of key physiological traits to breeding programs could help alfalfa breeders in indirect selection for WUE and forage production. Carbon isotope discrimination (Δ) has been proposed as a method and technique for evaluating and improving water use efficiency in C_3 plants like alfalfa (Ehleringer *et al.* 1993; Johnson and Tieszen 1994). The effectiveness of using any physiological criterion such as Δ in the selection process and breeding program depends on the magnitude of its association with the target trait (like WUE), the stability of this association in different environments, its genotypic variation and heritability, and finally the ease and cost of its measurement.

The physiological basis of the association between Δ and WUE is well established. WUE may be estimated as the ratio of dry matter accumulation over time to amount of water transpired (transpiration efficiency, TE) or as the ratio of CO_2 assimilation to stomatal conductance or transpiration (WUE of gas exchange or instantaneous WUE). In C_3 species, the isotopic ratio of heavy isotope of carbon (^{13}C) to ^{12}C in plant materials is less than the isotopic ratio of ^{13}C to ^{12}C in the atmosphere, indicating that plants discriminate against ^{13}C during photosynthesis which leads to a depletion of the plant dry matter in ^{13}C. This process depends on the ratio of the intercellular to atmospheric CO2 concentration (Ci/Ca) which is linked to stomatal conductance (Farquhar *et al.* 1982). Increasing CO2 assimilation or decreasing stomatal conductance results in increasing WUE and declining of leaf intercellular CO2 (C_i) and consequently Δ. Therefore, there should be a negative relationship between WUE and Δ due to the independent relation between C_i and Δ or WUE (Farquhar *et al.* 1982; Farquhar and Richard 1984). For breeding programs, the variation in the CO_2 assimilation to stomatal conductance or water transpiration ratio can be exploited in indirect selection for WUE via Δ. Johnson and Tieszen (1994) reported significant differences among 18 alfalfa accessions and a significant negative association between Δ and water-use efficiency in them. However, positive correlation between Δ and dry matter yield has been reported among nine alfalfa germplasms under irrigated condition (Ray *et al.* 1998) and also among 30 elite half-sib families grown in non stress and drought stress conditions (Ray *et al.* 1999a , 1999b). However, selection for low Δ has been suggested as a method to indirectly evaluate WUE and as a criterion to improve WUE in alfalfa breeding programs.

Different studies have also reported sufficient genetic variation and heritability for Δ in several crops including alfalfa (Johnson and Tieszen 1994; Ray *et al*., 2004), barley (Teulat *et al*., 2001) and cowpea (Ismail *et al*., 1994).

The Δ is easily measured by simple preparation of very small plant samples, and a large number of samples can be analyzed quickly and economically due to advances in mass spectrometry (Jefferies and Mackerron 1997).

The study of Δ in different plant parts might help breeders to choose the part which would maximize the correlation between Δ and WUE and its stability in different environments.

Stable and consistent genotype rankings for Δ in different shoot parts (Johnson and Rumbaugh 1995) and also different environments (Johnson and Tieszen 1994; Ray *et al.* 1998) in alfalfa suggest that any plant part can be sampled for evaluating Δ under either favorable or unfavorable conditions. Johnson and Rumbaugh (1995) reported that although plant parts (stem, upper leaves, bottom leaves and entire shoot) differed in Δ, there were similarities in Δ response across tested clones and water levels in plant parts. Johnson and Tieszen (1994) reported no genotype × environment interaction for Δ among 18 alfalfa accessions grown under irrigated and rain-fed conditions. Ray *et al*. (1998) also found no genotype × year interaction for Δ among 9 broad-based alfalfa germplasms under irrigated condition. On the contrary, Johnson and Rumbaugh (1995) reported significant clone × harvest interaction for Δ among 30 clones of alfalfa.

However, the most of these few studies on Δ in different plant parts have been focused on different parts of above-ground harvestable biomass in C_3 plants such as Johnson and Rumbaugh (1995) in alfalfa, Hannachi *et al.* (1996) in bread wheat and Hafsi *et al.* (2000) in durum wheat.

The objectives of this study were (i) to estimate WUE and Δ in different alfalfa genotypes under irrigated and rain-fed conditions, (ii) to determine the effect of drought stress on WUE and Δ under rain-fed condition, (iii) to study interrelationships between biomass productions, water use efficiency and carbon isotope discrimination in both conditions, and (iv) to examine variation and consistency of Δ in harvestable (shoot) and non-harvestable (stubble and root) parts of plant biomass under irrigated and rain-fed conditions.

Materials and Methods

Experiment description and design

In order to assess carbon isotope discrimination (Δ), relationship between WUE and Δ and the effect of drought stress in eighteen alfalfa genotypes (Table 2) containing eight Iranian ecotypes and ten European varieties in organic farming, this study was carried out in two separate trials, namely, irrigated (no water stress) and rain-fed (water stress) at two different organically managed fields, Gross-Enzersdorf (48º12' N, 16º33' E) and Raasdorf (48º15' N, 16º37' E), respectively, of the research station of the University of Natural Resources and Applied Life Sciences (BOKU), Vienna, Austria during 2006-08. The farm management was organic and stockless, no organic manures were applied. The soil in both fields is a Calcaric Phaeozem (WRB) from loess with a silty loam textur (Table 1).

Table 1. Some properties of the experimental soil at two field trials.

	Gross-Enzersdorf (Irrigated)	Raasdorf (Rain-fed)
Texture	Silty loam	Silty loam
Organic carbon content (%)		
0-30 cm	1.5	2.0
30-60 cm	1.4	0.7
Depth of A horizon	45-50cm	25-35cm
Bulk density (g cm^{-1})	1.4-1.6	1.3-1.4

The soils are described in detail in Freyer *et al.,* (2000) and Pietsch *et al.,* (2007). The amount of precipitation and applied irrigation water and the average temperate from March to September in 2007-8 are shown in Figure 1.

Raasdorf (Rain-fed) **2007** **Gross-Enzersdorf (Irrigated)**

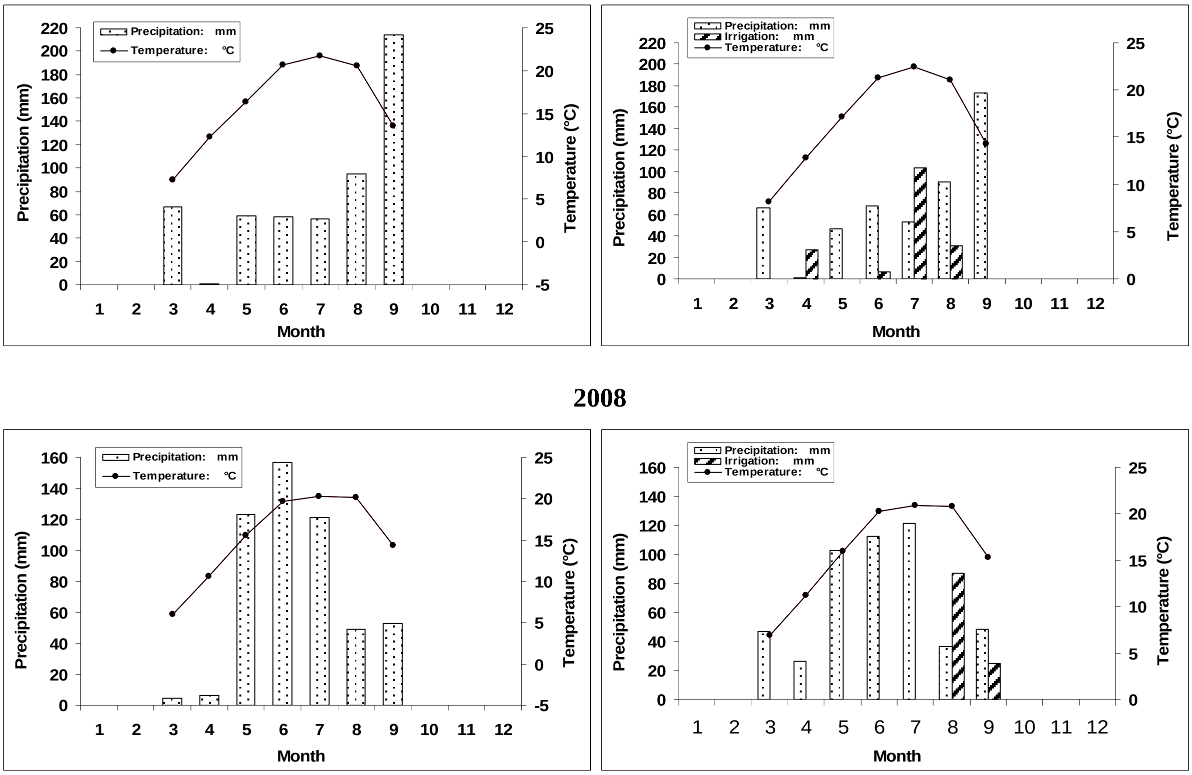


Fig. 1. Monthly precipitation, average temperature and applied irrigation water from March to September 2007 and 2008.

Both trials were hand seeded in May, 2006. The first experimental year (i.e. 2006) was considered as establishment year. During the establishment, plots were hand clipped one time in September 2006. The seeding density was 25 kg ha^{-1} which was adjusted by the germination rate of the cultivars. The experimental design was an α-lattice design with two replications. Each replication consisted of three incomplete blocks and each incomplete block consisted of six experimental alfalfa plots. Each genotype was seeded in 12 rows, each 1.5 m long, in the rain-fed trial at Raasdorf and 8 rows, each 1 m long, in the irrigated trial at Gross-Enzersdorf. Spacing between rows in both trials was 12.5 cm.In the irrigated trial, soil moisture content was monitored weekly by four FDR (Frequency Domain Reflectometry, ThetaProbe ML2x, UMS GmbH, München, Germany) probes in 15, 40, 80 and 120 cm soil depths; these devices were installed in one plot in each incomplete block including cultivars of 1, 9 and 18 in each replication while, in rain-fed trial, probes of SENTEK diviner 2000

Table 2. Name of tested genotypes and their origin.

No.	Name	Origin	No.	Name	Origin
1	Mohajeran	Iran-West	10	Verko	Hungry
2	Khorvande	Iran-West	11	Vlasta	Czech Republic
3	Famenin	Iran-West	12	Monz42	Slovakia
4	Ghargholgh	Iran-Northwest	13	Fix232	Slovakia
5	Ordobad	Iran-Northwest	14	NS- Banat	Serbia
6	Shorakat	Iran-Northwest	15	Sanditi	Netherlands
7	Ghara-aghaj	Iran-Northwest	16	Alpha	Netherlands
8	Hokm-abad	Iran-Northwest	17	Plato	Germany
9	Sitel	Netherlands	18	Niva	Czech Republic

Both trials were hand seeded in May, 2006. The first experimental year (i.e. 2006) was considered as establishment year. During the establishment, plots were hand clipped one time in September 2006. The seeding density was 25 kg ha^{-1} which was adjusted by the germination rate of the cultivars. The experimental design was an α-lattice design with two replications. Each replication consisted of three incomplete blocks and each incomplete block consisted of six experimental alfalfa plots. Each genotype was seeded in 12 rows, each 1.5 m long, in the rain-fed trial at Raasdorf and 8 rows, each 1 m long, in the irrigated trial at Gross-Enzersdorf. Spacing between rows in both trials was 12.5 cm.In the irrigated trial, soil moisture content was monitored weekly by four FDR (Frequency Domain Reflectometry, ThetaProbe ML2x, UMS GmbH, München, Germany) probes in 15, 40, 80 and 120 cm soil depths; these devices were installed in one plot in each incomplete block including cultivars of 1, 9 and 18 in each replication while, in rain-fed trial, probes of SENTEK diviner 2000 FDR system were installed in 2 plots (one tube in each plot) in each incomplete block including cultivars 1, 5, 9, 11, 14 and 18 to a soil depth of 120cm. Irrigation would be started at 50 % depletion of soil available water (SAW) content (SAW = water content difference between field capacity and permanent wilting point) based on FDR probe readings in 15 cm soil depth in the irrigated trial. The amount of applied irrigation water was calculated for 0-30 cm depth based on soil water content up to field capacity. Plots were irrigated by a drip irrigation system.

Data collection

Plots were hand clipped three times at 30-40 % of flowering using a garden scissor to a 5-cm stubble height every year (2007-08). Shoot (SHDM) and stubble (STDM) yield data (t ha^{-1}) were adjusted to a dry matter basis by sub-sampling approximately 200 and 50 g of fresh

shoot and stubble, respectively, from 0.5 m^2 of the plots at each harvest, and drying the samples at 60 °C for 48 h. Annual shoot dry matter production was determined by summing the yield data over the harvests within each year. Root dry matter (RODM) (t ha^{-1}) was determined using a soil corer with 9 cm diameter. Two samples were taken per plot down to 30 cm depth, and fresh roots after washing were dried at 60 °C for 48 h. Root dry matter and stubble dry matter were recorded only at the third harvest in each year.
Water use efficiency (WUE) was calculated based on both shoot dry matter (WUE-SHDM) and total biomass yield (WUE-TBY) as follows:

$$\text{WUE} = \text{Dry matter production / total water use [kg m}^{-3}\ \text{H}_2\text{O]}$$

Total water use during vegetation period was calculated according to an estimate of the actual evapotranspiration (ET) using the climatic water balance equation (Pietsch *et al*., 2007) as follows:

$$N + B = T + E + A + S + \Delta R$$

Where N, B, T, E, A, S and ΔR are precipitation, irrigation, transpiration, evaporation, surface runoff, leaching and change in the water content of the soil profile (0-90 cm), respectively. Surface runoff (A) and leaching (S) were considered as zero during the growth period. The change in the soil water content (ΔR) was calculated by weekly data recording from FDR probes under both conditions. The average of calculated ΔR in 2 plots of each incomplete block under rain-fed condition and calculated ΔR in single plot in each incomplete block under irrigated condition were used to adjust total water use of plots within an incomplete block. The total amount of applied water was determined for the rain-fed trial based on total precipitation and for irrigated trial based on summing up total precipitation and applied irrigation water during the vegetation period. Finally, the following simplified equation was used for calculating WUE:

$$T + E = N + B - \Delta R$$

Carbon isotope discrimination (Δ) in the various plant parts (shoot, stubble and root) was determined at harvest time each year. Each sample was sequentially ground in a 1-mm sieve and mixed extensively. The Δ values (‰) were determined with an isotope ratio mass spectrometer (IRMS-Thermo Quest Finnigan DELTA plus) in the laboratory of the

Department of Chemical Ecology, University of Vienna, according to procedures of Farquhar *et al.* (1989):

$$\Delta = \frac{\delta^{13}C_{air} - \delta^{13}C_{plant}}{1 + \delta^{13}C_{plant}}$$

where $\delta^{13}C$ is the value of stable isotope ratio (air or plant) which is expressed as the $^{13}C/^{12}C$ ratio (R_{sample}) relative to the PeeDee belemnite standard ($R_{standard}$) (Craig 1957):

$$\delta^{13}C(‰) = \left(\frac{R_{sample}}{R_{standard} - 1} \right) \times 1000$$

$R_{sample} = \frac{^{13}C}{^{12}C}$, $R_{standard}$ = -8 ‰

Statistical analysis

The data was subjected to three kinds of analysis of variance (ANOVA). All ANOVA was done by PROC MIXED in SAS software (SAS Institute, 2004). A SAS macro was used to find a letters display for all pairwise mean comparisons (Piepho, 2009).

Analysis of variance for individual harvest dates

The data of Δ-shoot from each harvest was individually analyzed based on alpha lattice analysis of variance, where incomplete block within replication [iblock (rep)] was considered as random and genotype and replication as fixed factors. All pairwise comparisons of adjusted mean of genotypes were done at 0.05 probability level.

Analysis of variance in each location

To determine difference among harvests and genotype by harvest interaction, data from six harvests of Δ-shoot (3 in first and 3 in second year) in each location were analyzed using repeated measure analysis of variance based on an alpha-lattice design. Replication (Rep) and genotype (G) were considered as fixed effects, while incomplete block within replication [iblock (rep)] and harvest (H) were considered as random effect and repeated measure, respectively, and the first-order autoregressive [AR (1)] of covariance structure was used in analysis. All pairwise comparisons of harvest means were done at 0.05 probability level. Mean comparisons were adjusted for the *p*-values ($\alpha = 0.05$) using ADJUST=SIMULATION option.

Combined analysis of variance across locations and years

Two years and locations data of WUE-SHDM, WUE-TBY, Δ-stubble, Δ-root and average of Δ-shoot over harvests per year were analyzed using repeated measure analysis of variance based on an alpha-lattice design. Location (L), replication (Rep) and genotype (G) were considered as fixed effects, while incomplete block into replication [iblock (rep)] and year (Y) were considered as random effect and repeated measure, respectively. The analysis was done using two different covariance structures, the unstructured (UN) and the first-order autoregressive [AR (1)]. The Akaike Information Criterion (AIC) was used to find the best model describing the covariance structure. Difference between Iranian ecotypes and European cultivars was evaluated by contrast using CONTRAST statement. Mean comparisons were adjusted for the *p*-values ($\alpha = 0.05$) using ADJUST=SIMULATION option.

Phenotypic correlation

Phenotypic correlations based on adjusted means of genotypes (n=18) for Δ-shoot were calculated between different harvests (6 harvests, 3 in the first and 3 in the second year) to evaluate relationships between harvests in each location. In addition, phenotypic correlations were calculated between SHDM, STDM, RODM, TBY, WUE-SHDM, WUE-TBY and Δ-shoot, Δ-stubble and Δ-root based on both adjusted means of genotypes over years in each location and over years and locations to examine relationships between traits and the usefulness of Δ of different plant parts as a selection tool for WUE improvement under different conditions.

Results

Under irrigated condition, genotypes did not differ at the first harvest-2007 and the third harvest-2008, while other harvests showed significant genotype differences at different probability levels (Table 3). The genotypes at the second harvests in 2007 and in 2008 differed significantly for Δ-shoot at 0.05 and 0.01 probability levels, respectively. Under rain-fed condition, significant differences ($P < 0.1$) occurred only at the third harvest of the second study year (2008).

Table 3. Significance levels for the Δ-shoot of genotypes at different harvests and conditions.

	Irrigated						Rain-fed					
	Harvest-2007			Harvest-2008			Harvest-2007			Harvest-2008		
Source	1^{st}	2^{nd}	3^{rd}	1^{st}	2^{nd}	3^{rd}	1^{st}	2^{nd}	3^{rd}	1^{st}	2^{nd}	3^{rd}
Genotype	ns	*	+	+	**	ns	ns	ns	ns	ns	ns	+
CV%	1.88	1.02	0.77	1.15	0.87	1.06	1.78	2.47	1.59	1.25	1.32	1.28

**, *, + and ns indicate significance level at 0.01, 0.05, 0.1 and non significant, respectively.

Table 4. Significance levels for the sources of variation for Δ-shoot in the combined ANOVA of six harvests of each location.

Source of variation	df	Irrigated	Rain-fed
Replication(R)	1	*	ns
Genotype (G)	17	+	ns
Harvest (H)	5	**	**
RxH	5	**	**
GxH	85	*	ns

**, *, + and ns indicate significance level at 0.01, 0.05, 0.1 and non significant, respectively.

The genotypes differed significantly (P < 0.1) in Δ-shoot (average over six harvests) under irrigated condition, while no significant difference was observed under rain-fed condition (Table 4). The harvest means had significant differences (P < 0.01) under both conditions. The genotype by harvest interaction was only significant under irrigated (P< 0.05) conditions.

Under irrigated condition, all rank correlations between harvests were positive except between H2-2007 and H2-2008. The correlations were significant only between H3-2007 and all three harvests of 2008 (Table 8). Under rain-fed condition, despite non significant G × H interaction, most of the rank correlation coefficients were small and non significant. The first harvest was correlated insignificantly to other harvests in both years (Table 8). The rank correlations between the second harvest of the first year (H2-2007) and other harvests were negative (Table 8). Rank correlation was positive and significant between third harvest-2007 and second harvest-2008 (r = 0.71**) and third harvest-2008 (r = 0.65**) and also between second and third harvests of 2008 (r = 0.59**).

The genotype means for Δ-shoot at different harvests under irrigated and rain-fed conditions are shown in Table 5 and 6, respectively. Under irrigated condition, Plato ZC (21.12 ‰), Sanditi (21.69 ‰) and Gharghologh (22.97 ‰) at the first through third harvest of 2007, respectively and Niva (21.52 ‰), Famenin (22.30 ‰) and Verko (22.52 ‰) at the first through third harvest of 2008 had the lowest values of Δ-shoot (Table 5). The range of Δ-shoot was 1.30, 0.77 and 0.79 ‰ in 2007 and 1.03, 1.20 and 0.90 ‰ in 2008 at the first, second and third harvest, respectively.

Table 5. Mean of genotypes for Δ-shoot at different harvests under irrigated condition.

	Harvests- 2007						Harvests- 2008					
Genotype	1st		2nd		3rd		1st		2nd		3rd	
Mohajeran	22.41	c	22.40	eh	23.29	bcd	21.76	abc	22.90	bcd	22.99	ad
Khorvande	22.14	bc	22.44	fh	23.19	bcd	21.67	ab	22.85	bc	22.94	ad
Famenin	22.15	bc	22.27	cdegh	22.98	ab	22.26	bde	22.30	a	22.60	abc
Gharghologh	21.37	ab	21.71	ab	22.97	a	22.02	ad	22.90	bcd	22.92	ad
Ordobad	22.06	ac	22.35	egh	23.44	def	21.96	ae	22.95	be	23.14	bd
Shorakat	22.12	ac	22.25	bdegh	23.50	def	22.36	cde	23.30	defg	22.87	ad
Ghara-aghaj	21.75	ac	22.02	ah	23.38	cf	22.11	ad	23.45	fg	23.10	bd
Hokmabad	22.02	ac	21.75	ad	23.31	ace	22.29	cde	23.10	cefg	22.54	ab
Sitel	22.04	ac	21.81	ag	23.53	def	22.18	bde	23.35	efg	23.12	cd
Verko	21.77	ac	21.75	ac	23.30	ace	21.81	abc	23.05	bf	22.52	a
Vlasta	21.93	ac	21.89	ae	23.55	def	22.50	de	23.00	be	23.11	bd
Monz 42	21.69	ac	22.46	h	23.38	acf	22.38	cde	23.00	be	23.42	d
Fix 232	22.42	c	22.40	eh	23.76	f	22.19	bde	23.50	g	23.34	d
NS-Banat	22.01	ac	21.89	aef	23.73	ef	22.22	bde	23.15	cefg	23.35	d
Sanditi	22.01	ac	21.69	a	23.43	bef	22.07	ad	23.00	be	23.20	cd
Alpha	21.73	ac	22.04	ah	23.45	def	22.55	d	22.65	ab	23.21	cd
Plato ZS	21.12	a	22.11	ah	23.42	def	22.07	ad	22.90	bcd	22.92	ad
Niva	21.59	ac	21.89	ae	23.14	bcd	21.52	a	22.95	be	22.84	ad
Iranian	22.00		22.15		23.26		22.05		22.97		22.89	
European	21.83		21.99		23.47		22.15		23.06		23.10	

There is no significant difference between means in the same column with one common letter ($P < 0.05$).

Table 6. Mean of genotypes for Δ-shoot at different harvests under rain-fed condition.

	Harvests- 2007						Harvests- 2008					
Genotype	1st		2nd		3rd		1st		2nd		3rd	
Mohajeran	20.75	ab	20.25	ab	21.31	ab	21.82	ac	22.47	abc	22.22	ab
Khorvande	21.15	b	20.30	ab	21.55	ab	22.07	bcd	22.80	bd	22.52	ab
Famenin	20.45	ab	20.40	ab	21.02	a	22.04	bcd	22.55	bd	21.96	a
Gharghologh	20.85	ab	20.45	ab	21.34	ab	21.59	ab	22.20	b	21.87	a
Ordobad	20.45	ab	20.90	b	21.30	ab	21.99	bcd	22.77	bd	21.95	a
Shorakat	20.60	ab	20.55	ab	21.22	ab	21.89	bcd	22.26	ab	22.36	ab
Ghara-aghaj	20.70	ab	20.55	ab	21.67	ab	21.19	a	22.95	ad	22.51	ab
Hokmabad	20.35	a	20.70	ab	21.47	ab	21.84	ad	22.67	bd	22.32	ab
Sitel	20.70	ab	20.60	ab	21.02	a	21.99	bcd	22.66	bd	22.26	ab
Verko	21.05	ab	20.00	ab	21.51	ab	22.08	bcd	22.98	cd	22.23	ab
Vlasta	20.85	ab	19.75	a	22.01	b	21.92	bcd	23.12	d	23.22	c
Monz 42	21.10	ab	20.30	ab	21.53	ab	21.79	ac	22.61	bd	22.53	ac
Fix 232	21.15	b	20.15	ab	21.95	b	22.27	cd	23.20	d	22.72	bc
NS-Banat	20.80	ab	20.25	ab	21.33	ab	21.59	ac	22.56	bd	22.28	ab
Sanditi	20.45	ab	19.90	ab	21.54	ab	21.59	ab	22.80	bd	22.47	ab
Alpha	20.60	ab	19.90	ab	21.61	ab	22.48	d	22.63	bd	21.98	a
Plato ZS	20.55	ab	19.90	ab	21.60	ab	21.94	bcd	22.82	bd	22.15	ab
Niva	20.45	ab	19.85	ab	21.47	ab	21.94	bcd	22.87	bd	22.52	ac
Iranian	20.66		20.51		21.36		21.80		22.58		22.21	
European	20.77		20.06		21.56		21.96		22.82		22.44	

There is no significant difference between means in the same column with one common letter ($P < 0.05$).

Under rain-fed condition, Hokmabad (20.35 ‰) at the first, Vlasta (19.75 ‰) at the second, Famenin and Sitel (21.02 ‰) at the third harvest in 2007 and Ghara-aghaj (21.19 ‰) at the first and Gharghologh at the second and third harvest (22.20 ‰ and 21.87 ‰, respectively) in 2008 had the lowest Δ-shoot (Table 6). Except for the second harvest in 2007, the average of Δ-shoot was smaller for Iranian than for European genotypes.

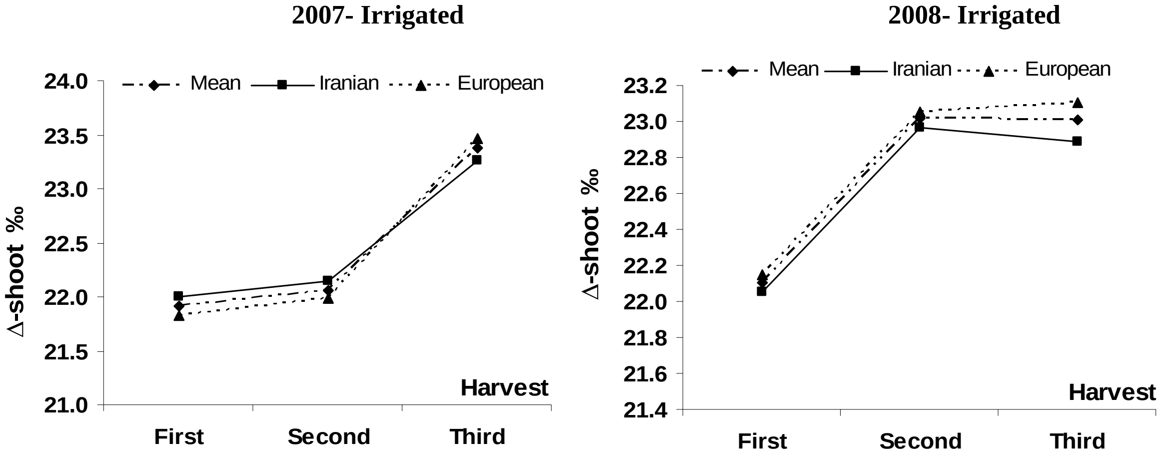


Fig. 2. Δ-shoot values for Iranian, European and grand mean of genotypes at different harvests under irrigated condition during 2007-08.

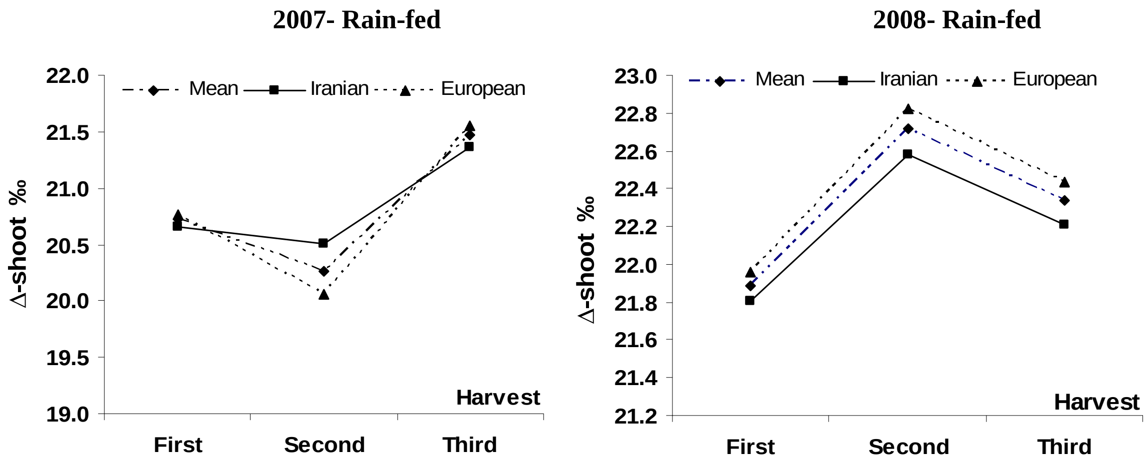


Fig. 3. Δ- shoot values for Iranian, European and grand mean of genotypes at different harvests under rain-fed condition during 2007-08.

The range of Δ-shoot, at the first to third harvest, was 0.8, 1.15 and 1.00 in 2007 and 1.29, 1.00 and 1.24 in 2008, respectively. Under irrigated condition, the lowest Δ- shoot belonged to the first harvest-2007 (21.9 ‰) and did not differ significantly from the second harvest in 2007 (22.1 ‰) and the first harvest in 2008 (22.1 ‰) (Table 7). Under rain-fed condition, the second harvest-2007 had the lowest response (20.3 ‰) which differed significantly from other harvests (Table 7).

Table 7. The harvest means comparisons for Δ- shoot under irrigated and rain-fed conditions

	Δ- shoot ‰			
Harvest	Irrigated		Rain-fed	
First (2007)	21.9	a	20.7	b
Second (2007)	22.1	a	20.3	a
Third (2007)	23.4	c	21.5	c
First (2008)	22.1	a	21.9	d
Second (2008)	23.0	b	22.7	f
Third (2008)	23.0	b	22.3	e
SE	0.05		0.07	

There is no significant difference between means in the same column with one common letter at 0.05 probability level.

Table 8. Rank correlations between Δ-shoot at different harvests under irrigated (Upper diagonal) and rain-fed (Lower diagonal) conditions.

	H1-2007	H2-2007	H3-2007	H1-2008	H2-2008	H3-2008
H1-2007		0.43	0.17	0.01	0.15	0.08
H2-2007	-0.22		0.11	0.03	-0.16	0.27
H3-2007	0.27	-0.58*		0.57*	0.54*	0.72**
H1-2008	0.17	-0.19	0.08		0.09	0.36
H2-2008	0.19	-0.41	0.71**	0.27		0.24
H3-2008	0.33	-0.33	0.52*	-0.11	0.56*	

* and ** are significant correlation at the 0.05 and 0.01 level, respectively (2-tailed).

H1, H2 and H3: first, second and third harvest, respectively.

The results of repeated measure analysis of variance for characters under study are shown in Table 9. The first order autoregressive (AR1) model of covariance structure was selected for WUE- SHDM and WUE- TBY based on the value of Akaike Information Criterion (AIC), while it was the unstructured model for Δ-shoot, Δ-stubble and Δ-root.

Table 9. Significance level for the fixed effects and their interactions in combined ANOVA of two locations.

Trait	WUE-SHDM	WUE-TBY	Δ-shoot	Δ-stubble	Δ-root
Source	kg m^{-3}	kg m^{-3}	‰	‰	‰
Location (L)	***	***	***	***	**
Year (Y)	***	***	***	***	**
Genotype (G)	***	***	*	ns	ns
Iranian vs. European	***	*	ns	ns	ns
L*Y	ns	ns	***	***	ns
L*G	***	***	ns	ns	ns
Y*G	**	**	ns	ns	ns
L*Y*G	***	**	ns	ns	ns

The significance levels 0.001, 0.01, 0.05 and non significant are indicated with ***, **, * and ns, respectively.

Locations (i.e. irrigated and rain-fed) and years showed highly significant differences in all characters. Average of WUE-SHDM and WUE-TBY was 3.0 and 4.8 kg m^{-3} under irrigated condition and 1.9 and 3.5 kg m^{-3} under rain-fed condition, respectively, showing a 34.4 % and 27.2 %-reduction due to drought stress effect under rain-fed condition (Table 11, Figs. 4 and 5). Drought stress under rain-fed condition also decreased the average values of Δ for shoot, stubble and root by -4.5, -4.0 and -5.3 %, respectively.

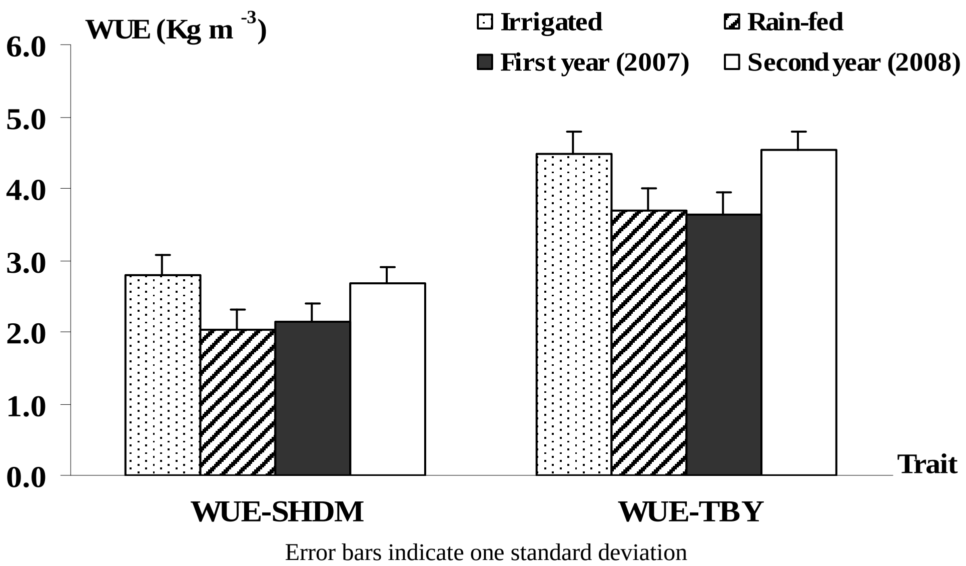


Fig 4. Mean of water use efficiency based on shoot dry matter (WUE-SHDM) and total biomass yield (WUE-TBY) for different locations (irrigated and rain-fed) and years.

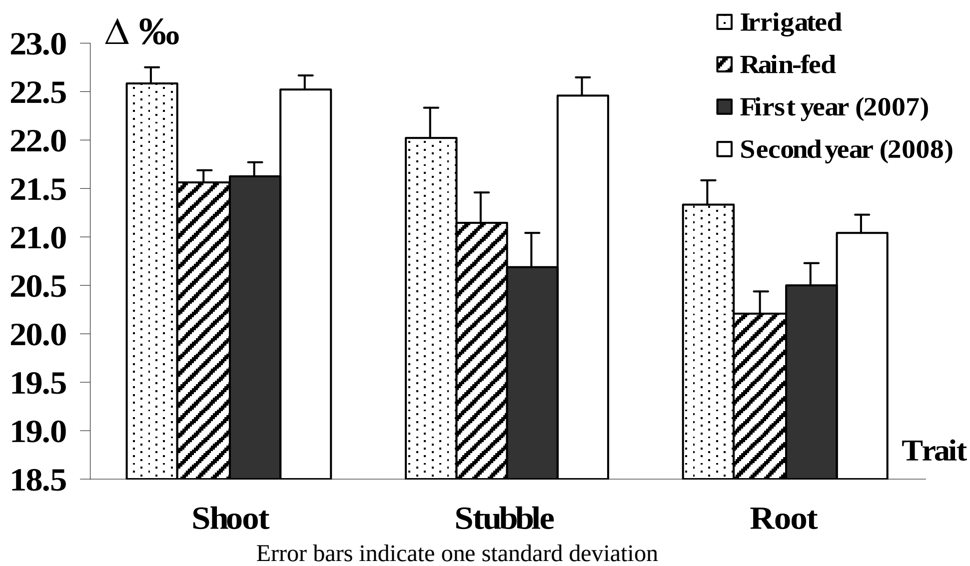


Fig 5. Mean of carbon isotope discrimination (Δ ‰) in shoot, stubble and root for different locations (irrigated and rain-fed) and years.

Average over locations for WUE-SHDM, WUE-TBY, Δ-shoot, Δ-stubble and Δ-root was 1.9 (kg m^{-3}), 3.2 (kg m^{-3}), 21.6 (‰), 20.7 (‰) and 20.5 (‰) in 2007 and 3.0 (kg m^{-3}), 5.1 (kg m^{-3}), 22.5 (‰), 22.5 (‰) and 21.0 (‰) in 2008, respectively showing 36.7, 37.3, 4.0, 7.9 and 2.6 % reduction in 2007 due to a different rainfall pattern and a higher intensity of drought stress (Figs. 4 and 5).

Significant differences among genotypes were found for WUE-SHDM, WUE-TBY and Δ-shoot, while no significant differences were observed for Δ-stubble and Δ-root (Table 9). However, phenotypic variation among genotypes was higher for WUE-SHDM and WUE-TBY than for Δ-shoot. Sitel had the highest WUE-SHDM (2.79 kg m^{-3}) and WUE-TBY (4.48 kg m^{-3}) across locations and years while Khorvande had the lowest mean with 1.82 and 3.70 kg m^{-3} for both characters, respectively (Table 10 and Fig. 6). Ghargholagh, Hokmabad and Plato ZS had the lowest Δ- shoot (21.87 ‰), Δ-stubble (21.11 ‰) and Δ- root (20.48 ‰) means over locations and years (Table 10 and Fig. 7). The overall mean for WUE-SHDM, WUE-TBY, Δ-shoot, Δ-stubble and Δ-root was 2.45 (kg m^{-3}), 4.14 (kg m^{-3}), 22.07 (‰), 21.57 (‰) and 20.77 (‰) with a range of 0.96 (kg m^{-3}), 0.78 (kg m^{-3}), 0.53 (‰), 0.80 (‰) and 0.56 (‰), respectively (Table 10).

Table 10. Comparisons of genotype means over years and locations for studied characters.

Trait	WUE-SHDM		WUE-TBY		Δ-shoot		Δ-stubble		Δ-root	
Cultivar	kg m^{-3}		kg m^{-3}		‰		‰		‰	
Mohajeran	2.59	ef	4.28	cde	22.02	ab	21.76	a	20.94	a
Khorvande	1.82	a	3.70	a	22.12	ab	21.41	a	21.05	a
Famenin	2.42	bce	4.05	ae	21.90	a	21.69	a	20.72	a
Ghargholagh	2.23	bc	4.25	cde	21.87	a	21.21	a	20.51	a
Ordobad	2.21	bd	3.97	abd	22.09	ab	21.25	a	20.73	a
Shorakat	2.46	bf	4.30	cde	22.14	ab	21.85	a	21.02	a
Ghara-aghaj	2.56	cf	4.12	ae	22.11	ab	21.69	a	20.83	a
Hokmabad	2.15	ab	3.96	abd	22.05	ab	21.11	a	20.62	a
Sitel	2.79	f	4.48	e	22.13	ab	21.75	a	20.94	a
Verko	2.56	cdf	4.11	ae	22.01	ab	21.82	a	20.68	a
Vlasta	2.64	ef	4.37	de	22.21	ab	21.84	a	20.75	a
Monz 42	2.30	bce	3.95	ad	22.21	ab	21.54	a	20.58	a
Fix 232	2.62	ef	4.39	de	22.40	b	21.91	a	20.91	a
NS-Banat	2.55	cdf	4.42	be	22.13	ab	21.69	a	21.05	a
Sanditi	2.53	cdf	4.02	ae	22.03	ab	21.65	a	20.61	a
Alpha	2.48	bf	3.85	ac	22.06	ab	21.59	a	20.79	a
Plato ZS	2.61	ef	4.14	ae	21.95	ab	21.15	a	20.48	a
Niva	2.63	ef	4.20	cde	21.91	a	21.41	a	20.70	a
Mean	2.45		4.14		22.07		21.57		20.77	
Range	0.96		0.78		0.53		0.80		0.56	
SE	0.09		0.12		0.10		0.25		0.20	

There is no significant difference at 0.05 level between genotypes with a common letter in the same column. SE = Standard error of mean.

Iranian and European genotypes did not show any significant difference for Δ in different plant parts while they differed significantly in WUE- SHDM and WUE-TBY (Table 9).

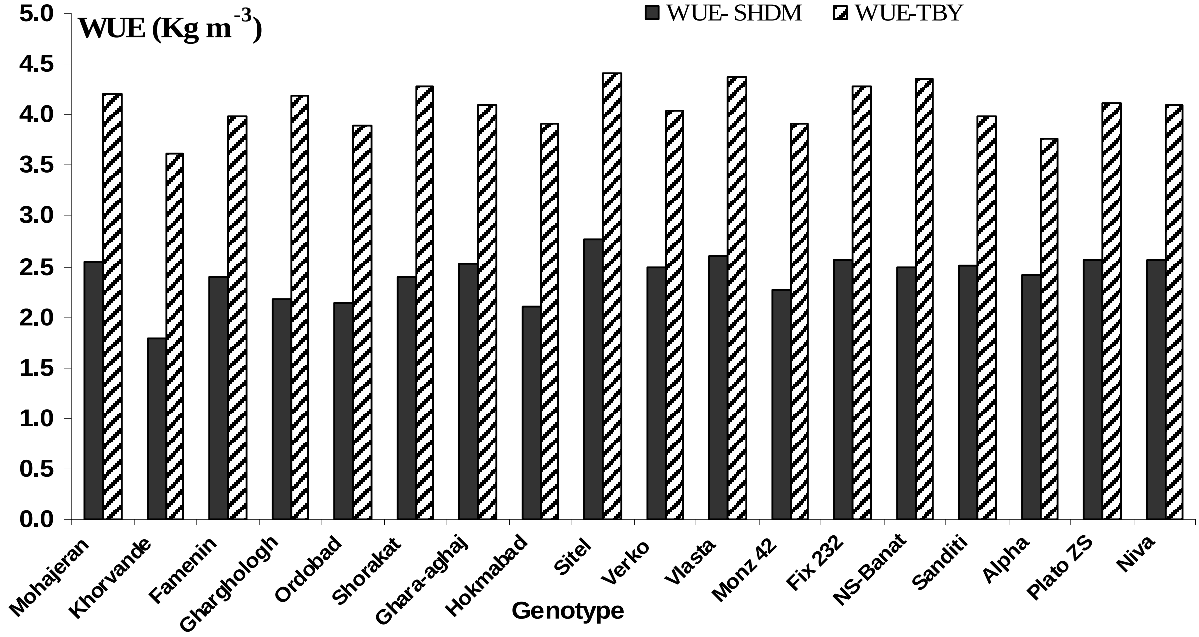


Fig. 6. Genotype means over years and locations of water use efficiency based on shoot dry matter (WUE- SHDM) and total biomass yield (WUE- TBY).

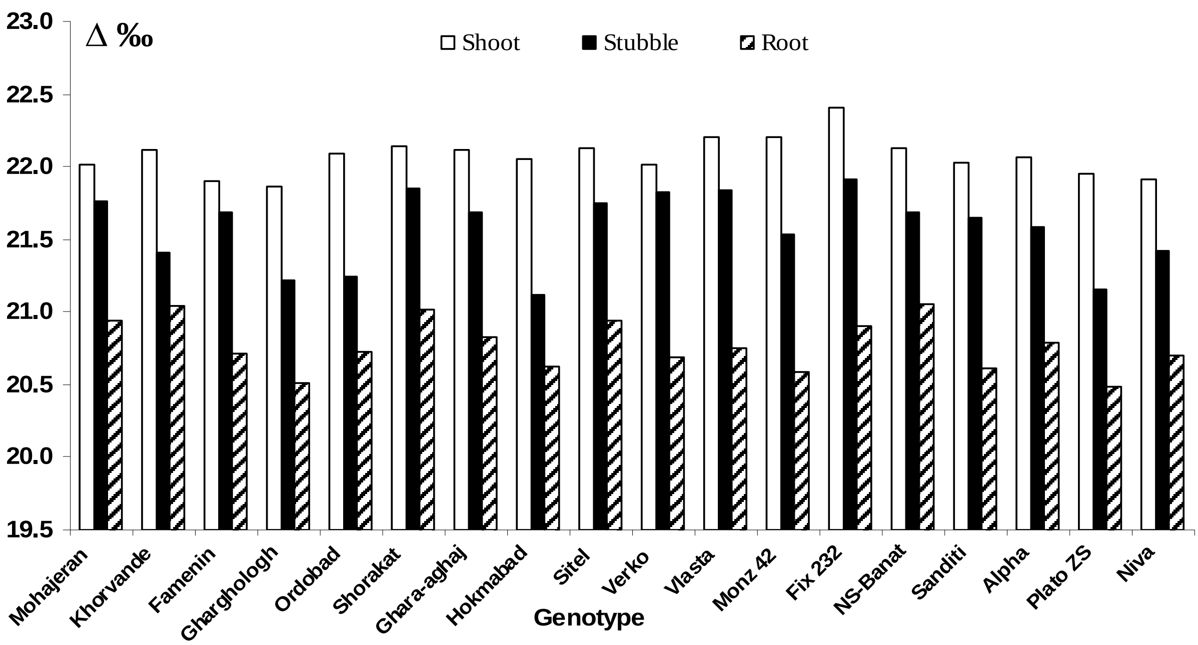
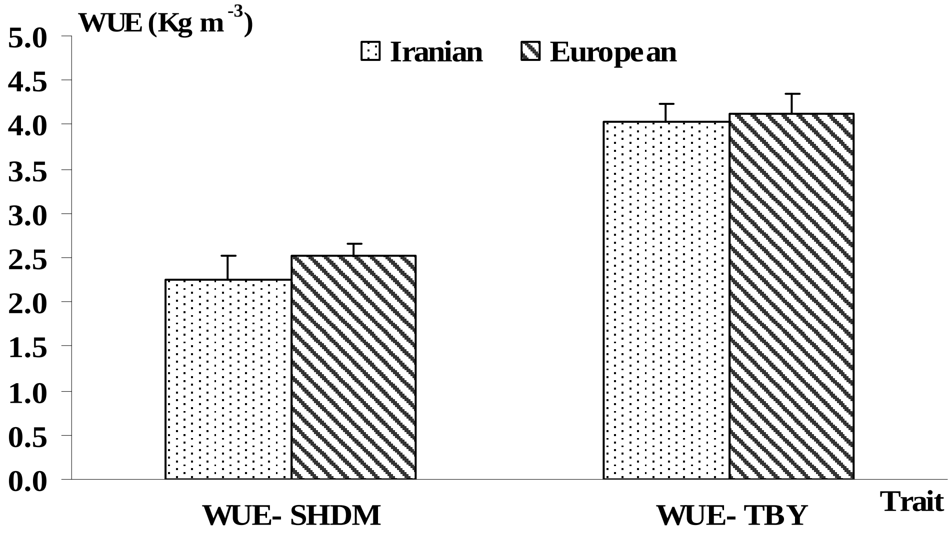


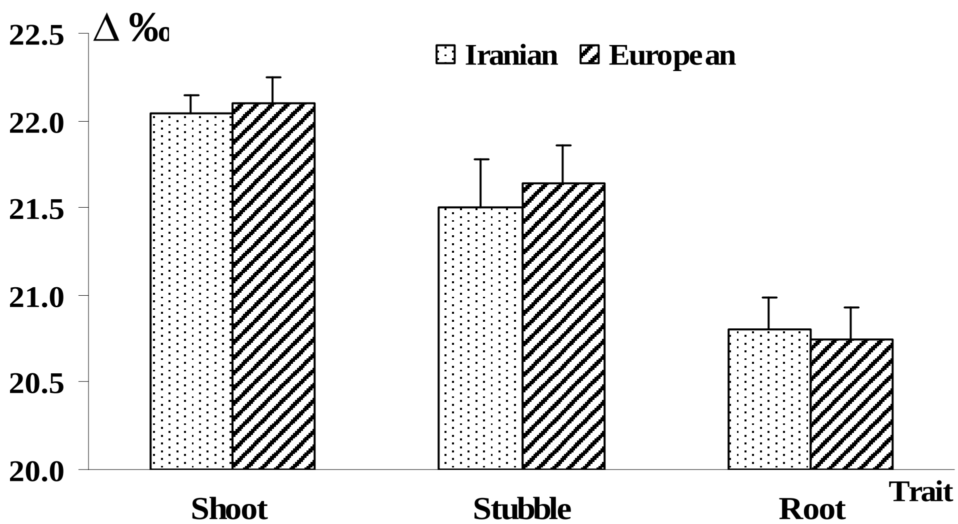
Fig. 7. Genotype means over years and locations of carbon isotope discrimination (Δ) in shoot, stubble and root.

The L × G, Y × G and L ×Y × G interactions were detected only for WUE-SHDM and WUE-TBY. However, the rank correlation between two locations based on genotype means over years for Δ-shoot, Δ-stubble and Δ-root was 0.37 (n= 18), 0.32 and 0.12, respectively. The rank correlation between the two study years within each location for Δ-shoot, Δ-stubble and Δ-root was 0.42 (n=18), 0.43 and 0.43 under irrigated condition and 0.13, 0.52* and 0.44 under rain-fed condition, respectively.



Error bar represents one standard deviation.

Fig. 8. Average of WUE-SHDM and WUE-TBY for Iranian and European genotypes.



Error bar represents one standard deviation.

Fig. 9. Average of Δ in various plant parts for Iranian and European genotypes.

Table 11. Genotype means over years in each condition including the percent of change for each genotype under rain-fed condition

	WUE-SHDM (kg m^{-3})				Change	WUE-TBY (kg m^{-3})				Change	Δ-shoot				Change	Δ-stubble				Change	Δ-root				Change
Cultivar	IR		RN		%	IR		RN		%	IR		RN		%	IR		RN		%	IR		RN		%
Mohajeran	3.4	e	1.8	ae	-47.2	5.1	bcd	3.4	ab	-33.6	22.6	a	21.4	a	-5.3	22.3	a	21.3	a	-4.6	21.8	a	20.1	a	-7.5
Khorvande	2.2	a	1.5	a	-31.1	4.4	ab	3.0	a	-31.9	22.5	a	21.7	a	-3.6	21.8	a	21.1	a	-3.3	21.3	a	20.8	a	-2.4
Famenin	2.9	bce	1.9	ae	-33.3	4.7	ad	3.4	ac	-28.0	22.4	a	21.4	a	-4.7	21.8	a	21.6	a	-1.0	21.2	a	20.3	a	-4.3
Ghargologh	2.6	ab	1.9	ae	-26.6	4.8	ad	3.7	ac	-23.5	22.3	a	21.4	a	-4.2	21.4	a	21.0	a	-1.7	20.9	a	20.1	a	-3.5
Ordobad	2.8	bcd	1.6	abd	-42.4	4.8	ad	3.1	ab	-35.2	22.6	a	21.6	a	-4.6	22.0	a	20.5	a	-6.8	21.4	a	20.0	a	-6.5
Shorakat	3.0	bce	1.9	ae	-36.4	4.8	ad	3.8	bc	-20.9	22.8	a	21.5	a	-5.7	22.4	a	21.3	a	-4.9	21.8	a	20.2	a	-7.1
Ghara-aghaj	2.9	bce	2.2	ce	-25.6	4.5	ab	3.8	bc	-15.6	22.6	a	21.6	a	-4.7	21.8	a	21.6	a	-1.0	21.4	a	20.3	a	-5.0
Hokm-abad	2.7	ac	1.6	ac	-39.5	4.7	ac	3.2	ab	-30.7	22.5	a	21.6	a	-4.1	21.6	a	20.7	a	-4.3	21.3	a	19.9	a	-6.6
Sitel	3.2	de	2.3	e	-28.4	5.1	bcd	3.8	bc	-25.3	22.7	a	21.6	a	-4.8	22.1	a	21.4	a	-3.4	21.5	a	20.4	a	-5.3
Verko	3.2	ce	1.9	ae	-42.4	5.0	ad	3.2	ab	-34.7	22.4	a	21.6	a	-3.4	22.5	a	21.2	a	-5.8	21.4	a	20.0	a	-6.4
Vlasta	3.1	bce	2.2	ef	-28.3	4.7	ac	4.1	c	-11.9	22.7	a	21.7	a	-4.0	22.1	a	21.5	a	-2.8	21.1	a	20.4	a	-2.9
Monz 42	2.9	bce	1.7	abcf	-40.5	4.7	ac	3.2	ab	-31.9	22.7	a	21.7	a	-4.6	22.1	a	20.9	a	-5.5	21.2	a	20.0	a	-5.5
Fix 232	3.2	de	2.0	cde	-36.5	5.5	d	3.3	ab	-39.6	22.9	a	21.9	a	-4.7	22.3	a	21.5	a	-3.5	21.4	a	20.4	a	-4.6
NS_Banat	3.0	bce	2.1	cde	-29.8	5.3	cd	3.6	ac	-32.5	22.7	a	21.5	a	-5.2	22.1	a	21.2	a	-4.0	21.7	a	20.4	a	-6.0
Sanditi	2.9	bce	2.2	ef	-23.3	4.3	a	3.7	ac	-14.3	22.6	a	21.5	a	-4.9	22.1	a	21.2	a	-4.3	21.2	a	20.0	a	-5.4
Alpha	3.2	ce	1.8	ae	-42.6	4.5	ab	3.2	ab	-30.3	22.6	a	21.5	a	-4.7	22.3	a	20.9	a	-6.2	21.3	a	20.3	a	-4.6
Plato ZS	3.1	ce	2.1	cde	-34.0	4.7	ac	3.6	ac	-24.2	22.4	a	21.5	a	-3.8	21.5	a	20.8	a	-3.0	21.0	a	20.0	a	-4.7
Niva	3.1	ce	2.1	be	-32.0	4.8	ad	3.6	ac	-24.9	22.3	a	21.5	a	-3.3	22.0	a	20.9	a	-5.1	21.4	a	20.0	a	-6.6
Mean	3.0		1.9		-34.4	4.8		3.5		-27.2	22.6		21.6		-4.5	22.0		21.1		-4.0	21.3		20.2		-5.3
Range	1.2		0.8			1.1		1.1			0.7		0.5			1.1		1.1			0.9		0.9		
SE	0.13					0.17					0.14					0.35					0.28				

IR =Irrigated, RN= Rain-fed and SE= Standard error of mean. Genotypes with a common letter in same column don’t differ significantly at 0.05 probability level.

Mohajeran with 3.4 kg m^{-3} under irrigated condition and Sitel with 2.3 kg m^{-3} under rain-fed condition exhibited the highest WUE-SHDM, and Khorvande with 2.2 kg m^{-3} under irrigated and 1.5 kg m^{-3} under rain-fed condition had the lowest WUE-SHDM (Table 11). The drought stress reduced overall the mean of WUE-SHDM by 34.4 % under rain-fed condition. Mohajeran with -47.2 % and Sanditi with -23.3 % had the greatest and the lowest reductions, respectively. Fix 232 with 5.5 kg m^{-3} and Vlasta with 4.1 kg m^{-3} exhibited the highest WUE-TBY under irrigated and rain-fed conditions, respectively, while Sanditi with 4.3 kg m^{-3} and Khorvande with 3.0 kg m^{-3} had the lowest WUE- TBY (Table 11). The overall mean of WUE-TBY was decreased by 27.2 % under rain-fed condition. Fix 232 with -39.6 % and Vlasta with -11.9 % exhibited the greatest and the smallest reduction, respectively (Table 11).

There was no significant difference in Δ- shoot among genotypes within each location (Table 11). Ghargholog and Niva with 22.3 ‰ under irrigated and Mohajeran, Famenin and Ghargholog with 21.4 ‰ under rain-fed condition had the lowest Δ- shoot responses. For Δ-stubble, the lowest values belonged to Ghargholog with 21.4 ‰ under irrigated and Ordobad with 20.5 ‰ under rain-fed condition. Ghargholog with 20.9 ‰ and Hokmabad with 19.9 ‰ had the lowest Δ-root responses among genotypes under irrigated and rain-fed conditions, respectively. There was a positive and significant correlation between WUE-SHDM and WUE-TBY based on genotype means under irrigated condition (r = 0.50*), rain-fed condition (r = 0.83**) and for the overall mean (r = 0.74**) (Table 12).

Table 12. Simple correlation between water use efficiency and Δ in different plant parts.

		WUE SHDM	WUE TBY	Δ Shoot	Δ Stubble
WUE-TBY	**IR**	0.53*			
	RN	0.83**			
	Mean	0.73**			
Δ-shoot	**IR**	0.25	0.36		
	RN	-0.06	-0.19		
	Mean	0.04	0.15		
Δ-stubble	**IR**	0.57*	0.36	0.56*	
	RN	0.55*	0.49*	0.11	
	Mean	0.58*	0.46*	0.33	
Δ-root	**IR**	0.37	0.47*	0.48*	0.64**
	RN	0.05	0.02	0.34	0.52*
	Mean	0.06	0.26	0.36	0.55*

IR= Irrigated, RN= Rain-fed, Mean = Overall mean; * and ** indicate significant correlations at 0.05 and 0.01 level, respectively (n = 18).

Low to intermediate positive correlations were observed between WUE-SHDM and Δ-shoot (r = 0.25), WUE-SHDM and Δ-root (r = 0.37), WUE-TBY and Δ-shoot (r = 0.36) and WUE-TBY and Δ-root (r = 0.47*) under irrigated condition. Negative correlations occurred between WUE-SHDM and Δ-shoot (r = -0.06) and WUE-TBY and Δ-shoot (r = -0.19), and negligible positive between WUE-SHDM and Δ-root (r = 0.05) and WUE-TBY and Δ-root (r = 0.02) under rain-fed condition (Table 12). There were positive and mostly significant correlations between Δ-stubble and WUE-SHDM and Δ-root and WUE-TBY under both conditions (Table 12). The correlations between Δ in different plant parts were positive and especially significant under irrigated conditions.

SHDM and Δ-shoot was correlated weakly and positive (r = 0.29) under irrigated while negative and negligible (r = -0.06) under rain-fed condition (Table 13).

Table 13. Simple correlations between water use efficiency, carbon isotope discrimination and biomass production under different conditions.

		WUE-SHDM	WUE-TBY	Δ-shoot	Δ-stubble	Δ-root
SHDM	IR	0.996**	0.55*	0.29	0.59*	0.41+
	RN	0.997**	0.82**	-0.06	0.57*	0.06
	Mean	0.998**	0.73**	0.05	0.59*	0.07
STDM	IR	0.11	0.43+	0.36	0.21	0.53*
	RN	0.19	0.05	-0.01	-0.23	-0.18
	Mean	0.21	0.26	0.14	-0.09	0.09
RODM	IR	-0.47*	0.49*	0.13	-0.17	0.12
	RN	0.02	0.58*	-0.20	0.08	-0.04
	Mean	-0.48*	0.24	0.16	-0.19	0.25
TBY	IR	0.53*	0.99**	0.39	0.38	0.52*
	RN	0.81**	0.997**	-0.18	0.49*	0.01
	Mean	0.71**	0.99**	0.18	0.45+	0.25

SHDM= Shoot dry matter, STDM= Stubble dry matter, RODM= Root dry matter, TBY= Total biomass yield;
+, * and ** indicate significant correlation at the 0.10, 0.05 and 0.01 probability level, respectively.

Stubble dry matter (STDM) and root dry matter (RODM) had positive correlations with Δ-stubble (r = 0.21) and Δ-root (r = 0.12), respectively, under irrigated condition, while negative correlations (r = -0.23 and r = -0.04, respectively) were observed under rain-fed condition.

Although WUE-TBY was intermediately and positively correlated with SHDM (r = 0.55, $P< 0.05$), STDM (r = 0.43, $P< 0.10$) and RODM (r = 0.49, $P< 0.05$) under irrigated condition, the correlation of these traits (SHDM, STDM and RODM) with and their contributions to WUE-TBY was different under rain-fed condition, the correlation between WUE-TBY and SHDM, STDM and RODM was 0.82**, 0.05 and 0.58*, respectively.

Discussion

The difference among genotypes for Δ-shoot was dependent on harvest time and stress condition (Table 3). Different significance levels among genotypes at different harvests of each location, especially under irrigated condition, indicate that the genotypes showed inconsistent differences in Δ-shoot. Pietsch *et al.* (2006) reported significant differences among 4 alfalfa cultivars for Δ-shoot only at the first harvest under organic rain-fed condition on the same experimental field. Three cultivars, Sitel, Vlasta and Verko, of the former experiment were included in this study.

Significant differences among genotype means over six harvests (three in the first and three in the second year) for Δ-shoot under irrigated condition (Table 4) exhibited differences in stomatal conductance and photosynthetic capacity among genotypes resulting in different assimilation rate and consequently different WUE and Δ responses of genotypes. It seems that drought stress under rain-fed condition reduced genotypic variation and/or increased environmental variation regarding the values of the coefficient of variation (CV %) (Table 3). Ray *et al*. (1998) stated that evaluating physiological and agronomic traits under irrigation may reduce the error variance relative to drought conditions where the influence of soil heterogeneity is magnified. However, the lower values of coefficient of variation across all harvests in both conditions (< 2.5 %) express a high precision in Δ- shoot assessment. Johnson and Rumbaugh (1995) reported an overall coefficient of variation less than 1 % for Δ and stated that the values of Δ are very reproducible across replications.

Significant difference among harvest means in both conditions indicated that the values of Δ-shoot differed by harvest so that the first harvest had the smallest value of Δ-shoot under irrigated condition in both years. Under rain-fed condition, however, the second harvest of the first year and the first harvest of the second year showed the lowest Δ-shoot values (Table 7 and Figs. 2 and 3). Pietsch *et al*. (2006) reported lower Δ-shoot mean for the second harvest (19.7 ‰) than for the first harvest (20.1 ‰) based on 4 cultivars on the same organic rain-fed experimental field. Johnson and Rumbaugh (1995) found the order of harvest 2 > harvest 3 > harvest 1 for Δ-shoot in an experiment on 30 alfalfa clones under different water levels.

Significant genotype by harvest interaction for Δ-shoot under irrigated condition (Table 4) indicated that the rank of genotypes changed between harvests and Δ-shoot response of genotypes was not stable and consistent across harvests under irrigated condition. Johnson and Rumbaugh (1995) also detected significant clone by harvest interaction in an experiment on 30 alfalfa clones under different water levels.

Despite non-significant genotype × harvest interaction under rain-fed condition, rank correlations between different harvests were low and mostly insignificant under both conditions (Table 8). An alfalfa cultivar is a heterogeneous population in which considerable genetic variation is observed for most morphological and physiological traits among individuals within each population. This variation along with the effect of environmental conditions like drought stress and different individual responses can be a reason for low and non-significant correlations between genotypes for Δ-shoot at different harvests. Therefore, caution should be taken when combining Δ-shoot data across harvests. Although there was no large difference in the amount of precipitation between two locations and years, mid- and late- season stresses were imposed on genotypes under rain-fed condition during the first and second year of the study, respectively (Fig. 1). Due to mid-season drought stress in 2007 (Fig. 1), H2-2007 was affected by drought stress more than other harvests and the Δ-shoot response of genotypes and consequently their ranks were changed by higher drought intensity in this harvest compared to other ones.

Although the difference between the average of Iranian and European genotypes was small, European genotypes showed higher Δ-shoot values except for the first and second harvest in 2007 (Fig. 2). One reason for lower Δ-shoot values under rain-fed than under irrigated conditions (Tables 5 and 6) can be less opening of stomata under rain-fed compared to irrigated conditions due to drought stress which resulted in a lower Δ-shoot under the rain-fed condition. The reduction of Δ-shoot mean values at the second harvest-2007 and the third harvest-2008 under rain-fed condition as harvests with high intensity of drought stress (Fig. 1) compared with the previous harvests, i.e. the first harvest-2007 and the second harvest-2008, respectively (Table 7 and Figs. 2 and 3), can also be regarded as an evidence for decreased Δ-shoot values under drought stress status due to closing stomata and decreasing stomatal conductance. Johnson and Rumbaugh (1995) reported lower values for Δ in the drought than in wet treatments in alfalfa clone experiments.

Regarding Δ- shoot values and ranges at different harvests, it can be concluded that the first harvest is the appropriate time to evaluate Δ-shoot response of genotypes for selection purpose under irrigated condition while under rain-fed condition, the suitable time should coincide with water stress which was the second harvest-2007 and the third harvest-2008. Generally, the first year of the study (2007) was the proper time to assess genotypes for their Δ- shoot response under both conditions regarding the Δ- shoot values and ranges, and also increasing the genetic gain and decreasing the costs due to a reduced time of each cycle of selection in alfalfa breeding programs.

Significant difference between two locations and years for WUE and Δ indicated the magnitude effect of drought stress under rain-fed condition (for source of location) and of different pattern of precipitation between two growing seasons (for source of year) on studied traits. In general, more severe drought stress under either rain-fed condition or in 2007, when the rainfall pattern during growing season caused more stress intensity than in 2008, reduced the overall mean of water use efficiency and carbon isotope discrimination responses, although the ratio of reductions differed between characters and genotypes.

Sitel and Khorvande were the best and the worst genotypes based on WUE-SHDM and WUE-TBY. However, the ranks of some top yielding genotypes such as Plato ZS and Niva and also low yielding genotypes such as Shorakat and Gharghologh changed when considering the total biomass, harvestable and non-harvestable, in estimation of WUE (WUE-TBY) compared with WUE-SHDM, indicating the importance of considering non-harvestable biomass, particularly roots, in a precise evaluation and selection of genotypes. Pietsch *et al.* (2006) found no significant differences among 4 alfalfa cultivars in WUE at the first and the second harvests on the same experimental field.

In spite of significant differences among genotypes (Table 9) and their diverse origin (Iranian and European genotypes), narrow ranges were found for all traits especially for WUE-TBY (0.78 kg m^{-3}) and Δ-shoot (0.53 ‰) based on genotype means over locations and years (Table 10). Increasing variation and ranges among genotypes for Δ and WUE under irrigated condition (Table 11) suggested more opportunities to improve WUE via Δ in this condition compared to rain-fed environment. Pietsch *et al*. (2006) reported 0.6 and 0.4 ‰ ranges among 4 alfalfa cultivars at the first and the second harvest under rain-fed condition, respectively.

Johnson and Rumbaugh (1995) reported that stems had the lowest Δ followed by entire shoot, upper leaves and bottom leaves. In our study, the stubbles as a short terminal part of stems showed lower Δ values than the entire shoot. The difference between below ground (roots) and above ground (shoots and stubbles) plant parts for Δ showed a general trend of an enrichment in ^{13}C in roots. Zhao *et al*. (2004) reported enrichment in ^{13}C and lower Δ in roots and grains compared to flag leaves and stems in two cultivars of upland rice during different developmental stages under three water regimes. Differences in chemical composition and anatomical characteristics between these plant parts can be caused by fractionating in export and partitioning of photosynthates among various plant parts (Brugnoli and Farquhar 2000). The average values of WUE-SHDM,WUE-TBY, Δ-shoot and Δ-stubble were higher and those of Δ- root were lower for European than for Iranian genotypes (Figs. 8 and 9). Based on Johnson and Rumbaugh (1995), a lower leaf-to-stem ratio in Iranian (0.72) than in European genotypes (0.83) (Table 6, chapter one) may cause the higher Δ-shoot values in European genotypes .

The non-significant L × G, Y × G and L ×Y × G interactions detected for Δ in various plant parts indicate no large rank changes and a consistent response of genotypes across diverse conditions in Δ in spite of significant effects of locations and years on these characters. Johnson and Tieszen (1994) found no genotype by environment interaction for Δ among 18 alfalfa genotypes grown under irrigated and rain-fed conditions. Thus, relatively stable ranking of alfalfa genotypes for Δ has been reported across different production environments (Johnson and Tieszen 1994; Johnson and Rumbaugh 1995; Ray *et al*., 1998). Ray *et al.* (1998) reported significant rank correlation r = 0.72 ($P<0.05$) between nine alfalfa germplasms across two years. In our study, in spite of non significant L × G, Y × G and L ×Y × G interactions, the rank correlation between two locations or years was positive and mostly non significant.

Although the rank correlation between genotype means under irrigated and rain-fed conditions was different and insignificant for WUE-SHDM (r = 0.36, n=18) and WUE-TBY (r = -0.26, n= 18), the effect of drought stress varied depending on genotypes under rain-fed condition. Some genotypes with high water use efficiency under irrigated condition such as Mohajeran, Fix 232 and Verko could not retain their superiority under rain-fed condition. In contrast, some genotypes with low water use efficiency under irrigated condition Such as Sanditi, Vlasta and Ghara-aghaj relatively improved their efficiency under rain-fed condition. In addition, the rank of some genotypes such as Sitel, Plato ZS, Niva, Khorvande, Hokmabad and Monz 42, either with high or low water use efficiency, was more or less the same under both conditions.

Regarding the genotype means for WUE-SHDM and WUE-TBY under both conditions (Table 11), Sitel was the most water use efficient genotype under both conditions (widely adapted genotype), followed by Mohajeran, Fix232 and Verko under irrigated condition (as specific adapted genotypes) and Vlasta, Sanditi, Ghara-aghaj under rain-fed condition. Pietsch *et al*. (2006) reported a high WUE of the cultivars Verko and Sitel, under rain-fed condition.

The lower reduction of WUE-TBY than WUE-SHDM due to drought stress under rain-fed condition (Table 11) implies that the non harvestable plant parts, particularly roots, were affected less by unfavorable conditions than the harvestable part (shoot). A higher correlation between WUE-SHDM and WUE-TBY under rain-fed condition (Table 12) can be regarded as a closer relation between harvestable and non-harvestable biomass under this condition.

The decrease of the overall mean of Δ under rain-fed condition was relatively similar in different plant parts (Table 11 and Fig. 5). However, the effect of drought stress on genotypes was more uniform for Δ-shoot than for Δ-stubble and Δ-root (Table 11). Jefferies and Mackerron (1997) reported that values for Δ-tuber in potato was consistently lower than stem and leaf and decreased more rapidly. Regarding the Δ response of genotypes in different plant parts, Iranian genotypes like Gharghologh had lower Δ values than European genotypes in most of the cases (Figs. 7 and 9). The relation between Δ-root and Δ-shoot was closer than the relation with Δ-stubble under both conditions (Table 12).

The correlation between SHDM and WUE-SHDM and between TBY and WUE-TBY was strong under both conditions (Table 13). The relation of SHDM and TBY and consequently water use efficiency (WUE-SHDM and WUE-TBY) with Δ responses of genotypes (shoot, stubble and root) were variable depending on plant part and environmental conditions (irrigated and rain-fed) (Tables 12 and 13). Correlations between Δ-shoot and shoot dry matter, total biomass yield and their relevant water use efficiency were positive under irrigated condition, while they were negative under rain-fed condition. Some high-yielding and water efficient genotypes such as Sitel, Fix 232, Vlasta (Tables 10 and 11) had high values of Δ-shoot reflecting greater assimilation and carbon fixation rate and consequently greater stomatal conductance in these genotypes than others. Pietsch *et al*. (2006) found no correlation between WUE and Δ-shoot based on 4 alfalfa cultivars under rain-fed condition. Johnson and Tieszen (1994) reported a significant negative correlation (r = -0.63 to -0.73) between shoot WUE and Δ among 18 alfalfa genotypes. Ray *et al.* (1998) found positive and significant correlation (r = 0.64, $P < 0.10$) between Δ and alfalfa shoot dry matter yield under irrigated condition. Ray *et al.* (2004) reported that higher yielding populations tended to have higher Δ in a diallel analysis among nine alfalfa germplasms. Read *et al.* (1991) in crested wheatgrass and Condon *et al.* (1987) in wheat reported a positive correlation between Δ and dry matter production, but Raeini-Sarjaz *et al* (1998) found a negative correlation (r = -0.88 to r = -0.92) between WUE and Δ in bush bean.

Regarding the correlation between SHDM, STDM, RODM and their relevant Δ under different conditions (Table 13), it can be concluded that the sign of relation between biomass production and Δ was changed from positive to negative by drought stress under rain-fed condition.

The SHDM was a better indicator of TBY and WUE-TBY, especially under rain-fed condition, compared to other components of TBY (STDM and RODM). Considering the results of mean comparisons based on WUE-SHDM and WUE-TBY (Tables 10 and 11) and correlations under irrigated condition (Table 13), TBY and WUE-TBY can be recommended for evaluating, ranking and selecting of high yielding and water efficient genotypes.

Regarding the positive correlation between different plant parts for Δ (Table 12), relationships between Δ responses and biomass production and water use efficiency under different conditions (Tables 12 and 13), and the required time and labor to assess individual plant parts, the Δ-shoots values may be the simplest and most economical single criterion to characterize alfalfa genotypes for high WUE via Δ in alfalfa breeding programs.

Based on our results about correlations between Δ-shoot, WUE-SHDM and WUE-TBY under different conditions, using Δ-shoot as the only selection criterion to improve WUE may cause some reductions in SHDM or TBY and consequently in WUE-SHDM or WUE-TBY depending on selected genotypes. The simultaneous assessment of genotypes for Δ-shoot and biomass production, however, can ensure selection of superior genotypes and minimize potential biomass reductions.

Based on simultaneous consideration on WUE-SHDM, WUE-TBY and Δ-shoot values (Table 10), Plato ZS and Niva from European genotypes and Gharghologh and Mohajeran from Iranian genotypes exhibited low values of Δ-shoot and acceptable WUE across locations and years. Due to opposite relations between WUE and Δ-shoot and differing variation and ranges of these traits under irrigated and rain-fed conditions, genetic gain and even selected genotypes may differ depending on a selection under either irrigated or rain-fed conditions. Narrow Δ variation among genotypes can be a reason for weak and insignificant correlation between Δ and WUE and biomass production in this study. So, hybridization and crossing between genotypes with high WUE such as Sitel, Vlasta and Fix 232 and genotypes with low Δ-shoot value such as Ghargholgh, Famenin, Plato ZS and Niva may create more variation and provide opportunities to improve simultaneously WUE and Δ-shoot. In addition, regarding the considerable genetic variation within alfalfa populations for different characters such as Δ (Johnson and Rumbaugh 1995) and WUE (Cole *et al.*,

1970) and the significant contribution of general combing ability (GCA) detected among different crosses (Johnson and Rumbaugh 1995; Ray *et al.*, 2004), based on our results, the Δ-shoot and WUE can be manipulated and improved by traditional alfalfa breeding techniques at the population level using Plato ZS, Niva , Ghargholegh, Sanditi, Sitel and Vlasta as initial selection germplasms.

Finally, It can be concluded that Sitel was the most water use efficient genotype across two condition (widely adapted genotype) followed by Mohajeran, Fix232 and Verko under irrigated condition (as specific adapted genotypes) and Vlasta, Sanditi, Ghara-aghaj under rain-fed condition. The overall mean of water use efficiency and carbon isotope discrimination responses were reduced due to drought stress, although the ratio of reductions differed for plant characteristics and genotypes. The average values of WUE- SHDM, WUE-TBY, Δ-shoot and Δ-stubble were higher and those of Δ- root were lower in European than in Iranian genotypes. Narrow ranges were found for all traits especially for WUE-TBY (0.78 kg m^{-3}) and Δ-shoot (0.53 ‰) based on genotype means over locations and years, although variation and ranges were higher under irrigated condition. The entire shoots were the best and additionally simplest and most economical single criterion to characterize alfalfa genotypes for high WUE via Δ in this study. The genotypes differed for Δ-shoot depending on harvest time and conditions indicating inconsistent differences in Δ-shoot. The rank correlations between different harvests for Δ-shoot were low and mostly insignificant under both conditions. Based on our results, the first harvest was the appropriate time to evaluate Δ-shoot response of genotypes for selection purpose under irrigated condition while under rain-fed condition, the suitable time should coincide with water stress. This was the second harvest-2007 and the third harvest-2008 in our study. Generally, the first year of our study (2007) was the proper time to assess genotypes for Δ- shoot response under both conditions.

The rank correlation between two locations or years was positive and mostly non-significant for Δ in all plant parts in spite of non significant L × G, Y × G and L ×Y × G interaction. Shoot and root samples had the highest and lowest Δ values, respectively. The association between SHDM and TBY and consequently water use efficiency (WUE-SHDM and WUE-TBY) with Δ responses of genotypes (shoot, stubble and root) were variable based on plant part and condition of study (irrigated and rain-fed). Correlations between Δ-shoot and shoot dry matter, total biomass yield and their relevant water use efficiency were positive under irrigated condition, while they were negative under rain-fed condition. Simultaneous assessment of genotypes for Δ-shoot and biomass production can ensure selection of superior genotypes and minimize potential biomass reductions that may occur when using Δ-shoot as only selection criterion to improve WUE. Among the European genotypes, Plato ZS and Niva, and among the Iranian genotypes, Ghargholegh and Mohajeran exhibited low values of Δ-shoot and acceptable WUE across locations and years.

Chapter Four

General conclusion

General conclusion

Based on the results of this study in the pervious three chapters, it can be generally concluded that:

1. The effects of year, location (irrigated and rain-fed), genotype and their interactions were significant for most of the studied characters.
2. Drought stress condition in the rain-fed trial reduced the average of all traits except for leaf to stem ratio, shoot and root protein content.
3. The cultivar Sitel was the best genotype (as widely adapted genotype) across two conditions. As for specific adaptation, Vlasta and Sitel can be selected for stress (rain-fed) and non-stress (irrigated) condition, respectively. Among Iranian ecotypes, Mohajeran under irrigated and Ghara-aghaj and Gharghologh under rain-fed condition had the best performance.
4. Although, AMMI analysis was found to be more informative in describing the adaptive response of the genotypes, P_i (superiority measure) was the best stability parameter to select high yield and stable genotype based on simplicity of calculation and correlation with crop performance in this study.
5. The high yielding genotypes had taller plants and denser stands, especially under rain-fed condition. Path analysis indicated all direct effects of BNF components were positive under both conditions, while some of the indirect effects were negative. Plant height and LAI can be considered as primary selection criteria for improving shoot DM, while crop regrowth and plant height, with antonymous effects, were more important for improving root dry matter.
6. Cluster analysis clearly differentiated Iranian ecotypes and European cultivars from each other using morphological and physiological data. However, under rain-fed condition, influence of drought stress and individual response of genotypes to drought stress resulted in small changes in grouping of genotypes. Regarding considerable genetic distance between Iranian and European genotypes in this study, different crossing programs can be planned between diverse genotypes to construct new varieties especially for organic farming systems.
7. The utility of different approaches for a breeding strategy for stress environments depends on stress intensity (SI). In low SI when the correlation between yield under stress and non-stress conditions is moderate to high and yield reduction due to stress condition is low, it seems the first approach (selection under good conditions and subsequent yield testing in stress environments) will work better than others. In high SI, when the correlation between

the yield under stress and non stress conditions is low and sometimes negative and yield losses due to stress condition is high, it seems the second approach (direct selection in target stress conditions) will work better than others. However, regarding the result of this study and others, the third approach, simultaneous evaluation of genotypes under both conditions, ensures the selection of superior genotypes for both conditions, especially in moderate stress intensity.

8. STI and GMP were the best indices to distinguish Group A genotypes from other groups, while SSI was the best one to separate genotypes with high yield potentials under stress condition.
9. The entire shoots were the best and additionally simplest and most economical single criterion rather than stubble and root to characterize alfalfa genotypes for high WUE via Δ in this study.
10. The genotypes differed for Δ-shoot depending on harvest time and conditions indicating inconsistent differences in Δ-shoot.
11. The rank correlations between different harvests for Δ-shoot were low and mostly insignificant under both conditions. Meanwhile, the rank correlation between two locations or years was positive and mostly non-significant for Δ in all plant parts in spite of non significant L × G, Y × G and L ×Y × G interactions.
12. The first year of our study (2007) was the proper time to assess genotypes for Δ-shoot response under both conditions. In addition, the first harvest was the appropriate time to evaluate Δ-shoot response of genotypes for selection purpose under irrigated condition while under rain-fed condition, the suitable time should coincide with water stress. This was the second harvest-2007 and the third harvest-2008 in our study.
13. The association between SHDM and TBY and consequently water use efficiency (WUE-SHDM and WUE-TBY) with Δ responses of genotypes (shoot, stubble and root) were variable based on plant part and condition of study (irrigated and rain-fed). Correlations between Δ-shoot and shoot dry matter, total biomass yield and their relevant water use efficiency were positive under irrigated condition, while they were negative under rain-fed condition. Thus, simultaneous assessment of genotypes for Δ-shoot and biomass production can ensure selection of superior genotypes and minimize potential biomass reductions that may occur when using Δ-shoot as only selection criterion to improve WUE.

References

-Abdul-Jabbar, A.S., D. G. Lugg, T. W. Sammis, and L. W. Gay. 1984. A field study of plant resistance to water flow in alfalfa. Agron. J. 76: 765–769.

-Akhtar, K., M. Ashraf, and A. Rao. 2000. Evaluation of selection indices for identification of productive mungbean *(Vigna radiata* L. Wilczek) genotypes under different water regimes. Pakistan Journal of Biological Sciences 3 (10): 1541-1544.

-Annicchiarico, P. 1997. Additive main effects and multiplicative interaction (AMMI) analysis of genotype-location interaction in variety trials repeated over times. Theor. Appl. Genet. 94: 1072-1077.

-Annicchiarico, P. 2002. Genotype x environment interactions: Challenges and opportunities for plant breeding and cultivar recommendations. p. 2-3. Available at: www.iao.florence.it/training/adaptation/refernces.php. FAO plant production and protection paper NO. 174. FAO, Rome.

-Annicchiarico, P. 2007a. Lucerne shoot and root traits associated with adaptation to favorable or drought-stress environments and to contrasting soil types. Field Crops Res. 102: 51-59.

-Annicchiarico, P. 2007b. Wide- versus specific-adaptation strategy for Lucerne breeding in northern Italy. Theor. Appl. Genet. 114: 647-657.

-Annicchiarico, P., L. Pecetti, L. DeRosa, and E. Piano. 2006. Lucerne variety evaluation for organic farming in northern Italy. In D. Rosellini and F. Veronesi (Eds.), Breeding and seed production for conventional and organic agriculture. Proceeding of XXVI meeting of the EUCARPIA fodder crops and amenity grasses section, XVI meeting of the EUCARPIA *Medicago* spp. group, 3-7 September, Perugia, Italy.

-Atlin, G. N., H. R. Lafitte, D. Tao, M. Laza, M. Amante, and B. Courtois.2006. Developing rice cultivars for high-fertility upland systems in the Asian tropics. Field Crops Research 97: 43–52.

-Avice, J.C., A. Ourry, G. Lemaire, J. J. Volenec, and J. Boucaud. 1997a. Root protein and vegetative storage protein are key organic nutrients for alfalfa shoot regrowth. Crop Sci. 37: 1187–1193.

-Barbercheck, M. E. 2009. Ecological Understanding of Insects in Organic Farming Systems: Diversity, Stability, and Productivity. Available online at: http://www.extension.org/article/18534.

-Barnes, D. K., B. P. Goplen, and J. E. Baylor. 1988. Highlights in USA and Canada, P. 1-24. In: A. A. Hanson, D. K. Barens, and R. R. Hill, Jr. (ed.), Alfalfa and alfalfa improvement, No. 29 in Agronomy Series. Amer. Soc. Agron. Madison, Wisc. USA.

-Bauchan, G. R, N. Diwan, and M. McIntosh. 1993. Development and evaluation of a core germplasm collection of arumal *Medicago* species in the United States. In: Proceedings of the 17th International Grassland Congress. New Zealand, Australia, 1: 265-266.
-Becker, H. B., and J. Leon. 1988. Stability analysis in plant breeding. Plant Breed. 101:1–23.
-Berg, W. K., S. M. Cunningham, S. M. Brouder, B. C. Joern, K. D. Johnson, J. B. Santini, and J. J. Volenec. 2007. The long-term impact of phosphorus and potassium fertilization on alfalfa yield and yield components. Crop Sci. 47: 2198-2209
-Betran, F.J., D. Beck, M. Banziger, and G. O. Edmeades. 2003. Genetic analysis of inbred and hybrid grain yield under stress and non-stress environments in tropical maize. Crop Sci. 43: 807–817.
-Blum, A. 1999. Towards standard assays of drought resistance in crop plants. In J. M. Ribaut and D. Poland (Eds.), Molecular Approaches for the Genetic Improvement of Cereals for Stable Production in Water-Limited Environments. Final report of workshop, 21-25 June, CIMMYT, Mexico.
-Boddey, R. M. 1987. Methods for quantification of nitrogen fixation associated with gramineae. CRC Critical Reviews in Plant Sciences 6: 209–266.
-Boller, B., P. Tanner, and F. X.. Schubiger. 2006. Discriminatory power of herbage yield trials under organic conditions. In D. Rosellini and F. Veronesi (Eds.), Breeding and seed production for conventional and organic agriculture. Proceeding of XXVI meeting of the EUCARPIA fodder crops and amenity grasses section, XVI meeting of the EUCARPIA *Medicago* spp group, 3-7 September, Perugia, Italy.
-Boyer, J. S. and H. G. McPherson. 1975. Physiology of water deficits in cereal crops. Adv. Agron. 27: 1-23.
-Boyer, J. S., and M. E. Westgate. 2004. Grain yields with limited water. Journal of Experimental Botany, 55 (407): 2385-2394.
-Brugnoli, E. and G. D. Farquhar. 2000. Photosynthetic fractionation of carbon isotopes, p. 352–434. In: R. C. Leegood, T. D. Sharkey, and S. von Caemmerer, (ed.), Photosynthesis: Physiology and Metabolism. Kluwer Academic Publishers, Dordrecht, the Netherlands.
-Brummer, E. C. 1999. Capturing heterosis in forage crop cultivar development. Crop Sci. 39: 943-954.
-Brunner, H., and F. Zapata. 1984. Quantitative assessment of symbiotic nitrogen fixation in diverse mutant lines of field bean (*Vicia faba minor).* Plant and Soil 82: 407-413.
-Buddenhagen, I. W. 1983. Breeding strategies for stress and disease resistance in developing countries. Ann. Rev. Phytopatho. 27: 385-409.

-Campiglia, E., F. Caporali, R. Barberi, and R. Mancinelli. 1999. Influence of 2-, 3-, 4-, 5-year stands of alfalfa on winter yield. In J.E. Olesen, R. Eltun, M.J. Goodling, E.S. Jensen, and U. Köpke (Eds.), Designing and Testing Crop Rotations for Organic Farming. Proceeding from an International Workshop, 165-171. DARCOF, Tjele, DK.
-Carnahan, H. L. 1960. Some theoretical considerations of the consequences of multiple alleles in relation to inbreeding and testing procedures in autopolyploids. Rep 17*th* Alfalfa Improv Conf., Saskatoon, Sask. Canada, pp. 19–22.
-Carter, P.R., and C. C. Sheaffer. 1983. Alfalfa response to soil water deficits. I. Growth, forage quality, yield, water use and water-use efficiency. Crop Sci. 23: 669-675.
-Casler, M., H. Riday, and D. Undersander. 2006. Organic vs. conventional fodder crops in the USA: A challenge for breeders? In D. Rosellini and F. Veronesi (Eds.), Breeding and seed production for conventional and organic agriculture. Proceeding of XXVI meeting of the EUCARPIA fodder crops and amenity grasses section, XVI meeting of the EUCARPIA *Medicago* spp group, 3-7 September, Perugia, Italy.
-Ceccarelli, S. 1987. Yield potential and drought tolerance of segregating populations of barley in contrasting environments. Euphytica 40: 197–205
-Ceccarelli, S. and S. Grando. 1991. Selection environment and environmental sensitivity in barley. Euphytica 57: 157–167.
-Chalk, P.M. and J. K. Ladha. 1999. Estimation of legume symbiotic dependence: an evaluation of techniques based on ^{15}N dilution. Soil Biol. Biochem. 31: 1901–1917.
-Clarke, J. M., R. M. De Pauw, and T. M. Townley-Smith. 1992. Evaluation of methods for quantification of drought tolerance in wheat. Crop Sci. 32: 728–732.
-Cole, D. F., A. K. Dobrenz, M. A. Massengale, and L. N. Wright. 1970. Water requirement and its association with growth components and protein content of alfalfa (*Medicago sativa* L.). Crop Sci. 27: 237-240.
-Condon, A. G., R. A. Richards, and G. D. Farquhar. 1987. Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field-grown wheat. Crop Sci. 27: 996–1001.
-Condon, A. G., R. A. Richards, G. J. Rebetzke, and G. D. Farquhar. 2004. Breeding for high water-use efficiency. Journal of Experimental Botany 55: 2447–2450.
-Craig, H. 1957. Isotopic standards for carbon and oxygen and correlation factors for mass spectrometric analysis of carbon dioxide. Geochim. Acta 12: 133-149.
-Crossa, J., H. G. Gauch, and R.W. Zobel. 1990. Additive main effects and multiplicative interaction analysis of two international maize cultivar trials. Crop Sci., 30: 493-500.

-Crossa, J., I. H. DeLacy, and S. Taba.1995. The use of multivariate methods in developing a core collection. In: T. Hodgkin, A. H. D. Brown, T. J. L. van Hintum and E. A.V. Morales (Eds), Core Collections of Plant Genetic Resources, pp. 77–92. J. Wiley & Sons, Chichester.

-Degenhart, N. R., D. K. Barnes, and C. P. Vance. 1992. Divergent selection for nodule aspartate aminotransferase and asparagine synthetase activities in alfalfa. Crop Sci. 32: 313-317.

-Dehghan-Shoar, M., J. G. Hampton, and M. J. Hill. 2005. Identifying and discriminating among lucerne cultivars using morphological characters. New Zeal. J. Agric. Res., 48: 271–276

-Demarly, Y. 1960. The use of inbreeding in the improvement of lucerne. Proc 8*th* Int Grassl Congress. Reading, Alden Press, Oxford, U.K. 8: 49–51.

-Dessureaux, L. 1976a. Increase of hybrid vigor in alfalfa by allelic selection. Forage notes 21: 22–26.

-Dessureaux, L. 1976b. Allelic selection – a first look at some integrated results. Forage notes 21: 26–27.

-Dessureaux, L. 1977a. Allelic selection in alfalfa. Forage notes 22: 2–9.

-Dewey, D. R., and K. H. Lu. 1959. A correlation and path coefficient analysis of components of crested wheat grass seed production. Agronomy J., 51: 515-518.

-Donovan, T. J., and B. D. Meek. 1984. Response of alfalfa to different levels of irrigation. Proceeding of 14th California Alfalfa Symposium. 5-6 December, Visalia, California

-Dunbier, M. W., and E. T. Bingham. 1975. Maximum heterozygosity in alfalfa: Results using haploid-derived autotetraploids. Crop Sci 15: 527–531.

-Ebdon, J. S., and H. G. Gauch, Jr. 2002. Additive main effect and multiplicative interaction analysis of national turfgrass performance trials: I. Interpretation of genotype x environment interaction. Crop Sci. 42: 489–496.

-Ehleringer, J. R., A. E. Hall, and G. D. Farquhar. 1993. Stable isotopes and plant carbon-water relations. Academic Press, San Diego, Calif.

-Falcinelli, M., and R. Torricelli. 2004. A new variety of lucerne for Italian organic farming. Proceeding of the first world conference on organic seed. July 5-7, FAO Head quarters, Rome, Italy.

-Farquhar, G. D. and R. A. Richards. 1984. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Aust. J. Plant Physiol. 11: 539-552.

-Farquhar, G. D., J. R. Ehleringer, and K. T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40: 503-537.

-Farquhar, G. D., M. H. O'Leary, and J. A. Berry. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust. J. Plant Physiol. 9:121-137.

-Farshadfar, E. 2008. Incorporation of AMMI stability value and grain yield in a single non-parametric index (GSI) in bread wheat. Pakistan J. of Biological Sciences. 11 (14): 1791-1796.
-Farshadfar, E. and J. Sutka. 2003. Multivariate analysis of drought tolerance in wheat substitution lines. Cereal Res. Commu. 31: 33-40.
-Fernandez, G. C. J. 1992. Effective selection criteria for assessing stress tolerance. In: Kuo, C.G. (Ed.), Proceedings of the International Symposium on Adaptation of Vegetables and Other Food Crops in Temperature and Water Stress, Publication, Tainan, Taiwan.
-Fick, G. W., D. A. Holt, and D. G. Lugg. 1988. Environmental physiology and crop growth. *In*: A. A. Hanson, D. K. Barens, and R. R. Hill, Jr. (ed.), Alfalfa and alfalfa improvement, No. 29 in Agronomy Series. Amer. Soc. Agron. Madison, Wisc. USA. pp. 163-194.
-Fischer, R. A. and R. Maurer. 1978. Drought resistance in spring wheat cultivars. Part 1: grain yield response. Aust. J. Agric. Res. 29: 897–912.
-Flores, F., M. T. Moreno, and J. J. Cubero. 1998. A comparison of univariate and multivariate methods to analyze G 3 E interaction. Field Crops Res. 56: 271–286.
-Fox, P.N., B. Skovmand, B. K. Thompson, H. J. Braun, and R. Cormier. 1990. Yield and adaptation of hexaploid spring triticale. Euphytica 47: 57–64.
-Frame, J., J. F. L. Charlton, and A. S. Laidlaw. 1998. Temperate Forage Legumes. CAB International, Wallingford, 327 pp.
-Francis, T. R., and L. W. Kannenberg. 1978. Yield stability studies in short-season maize: I. A descriptive method for grouping genotypes. Can. J. Plant Sci. 58: 1029–1034.
-Freyer, B., J. K. Friedel, C. Vogl, G. Pietsch, and O. Ehrmann. 2000. Monitoring von Bodenkennwerten in der Umstellung auf Ökologischen Landbau im Trockengebiet Ostösterreichs. Institut für Ökologischen Landbau, Universität für Bodenkultur Wien. 1-75. Wien. Abschlußbericht Forschungsprojekt Nr. 1170.
-Gabriel, K. R. 1971. The biplot graphic display of matrices with application to principal component analysis. Biometrika. 58: 453–467.
-Gauch, H. G. 1992. Statistical analysis of regional yield trials: AMMI analysis of factorial designs. Elsevier, Amsterdam, The Netherlands.
-Gauch, H. G. 2006a. Statistical analysis of yield trial by AMMI and GGE. Crop Sci. 46:1488-1500.
-Gauch, H. G., and R.W. Zobel. 1996. AMMI analysis of yield trials. *In* M.S. Kang and H.G. Gauch, Jr. (ed.) Genotype by environment interaction. CRC Press, New York. pp.85-123.
-Gauch, H. G. Jr., H. P. Piepho, and P. Annicchiarico. 2008. Statistical analysis of yield trials by AMMI and GGE: Further considerations. Crop Sci. 48: 866-889.

-Giauffret, C., J. Lothrop, D. Dorvillez, B. Gouesnard, and M. Derieux. 2000. Genotype x environment interactions in maize from temperate or highland tropical origin. Crop Sci. 40: 1004–1012.
-Giller, K. E. 2001. Nitrogen Fixation in Tropical Cropping Systems, 2nd/Ed. CAB International, Wallingford, 423 pp.
-Graham, P. H. 1992. Stress tolerance in *Rhizobium* and *Bradyrhizobium*, and nodulation under adverse soil conditions. Can. J. Microbiol. 38: 475-484.
-Graham, P. H., and J. C. Rosas. 1977.Growth and development of indeterminate bush and climbing beans associated with *Rhizobium*. J. Agric. Sci. Cambr. 88: 503-508.
-Graham, P. H., M. Hungria, and B. Tlusty. 2004. Breeding for better nitrogen fixation in grain legumes: Where do the rhizobia fit in. www.plantmanagementnetwork.org/pub/cm/review /2004/rhizobia/. Access on 10.06.2009.
-Guo, Z. G., H. X. Liu, Y. R. Wang, S. M. Wang, and G. D. Cheng. 2004. Suitability of lucerne cultivars, with respect to root development, to semi-arid conditions in west China. New Zealand Journal of Agricultural Research, Vol. 47: 51-59.
-Guo, Z., Z. Zhang, F. Hou, J. Xiao, and N. Lu. 2002. Study on root system development ability of several lucerne cultivars in hills and valleys of loess plateau. Chinese Journal of Applied Ecology 13: 1007–1012.
-Hafsi, M., P. Monneveux, O. Merah, and A. Djekoune. 2000. Carbon isotope discrimination and yield in durum wheat grown in the high plains of Setif (Algeria). Contribution of different organs to grain filling. Options Mediterraneennes, 40: 145—147.
-Halim, R. A., D. R. Buxton, M. J. Hattendorf, and R. E. Carlson. 1989. Water-stress effects on alfalfa forage quality after adjustment for maturity differences. Agron. J. 81: 189-194
-Hall, N. M., H. Griffiths, J.A. Corlett, H.G. Jones, J. Lynn, and G.J. King. 2005. Relationships between water-use traits and photosynthesis in *Brassica oleracea* resolved by quantitative genetic analysis. Plant Breeding 124: 557–564.
-Hallauer, A. R. and J. B. F. Miranda. 1988. Quantitative Genetics in Maize Breeding 2nd edn. Iowa State University Press, Ames.
-Hannachi, L., E. Deleens, and P. Gate. 1996. Nitrogen and carbon isotope composition of wheat grain: alteration due to sink-source modifications at flowering. Rapid Comm. Mass Spectrom., 19: 979—986.
-Hansen, A. P. 1994. Symbiotic N2 fixation of crop legumes. Margraf Verlag, Germany.248 pp.
-Hardy, R. W. F., R. C. Burns, and R. D. Holsten. 1973. Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil Biol. Biochem. 5: 47-81.

-Hill, R. R., J. S. Shenk, and R. F. Barnes. 1988. Breeding for yield and quality. *In*: A. A. Hanson, D. K. Barens, and R. R. Hill, Jr. (ed.), Alfalfa and alfalfa improvement, No. 29 in Agronomy Series. Amer. Soc. Agron. Madison, Wisc. USA. pp. 809-823.
-Hoeppner, J. W., M. H. Entz, B. G. McConey, R. P. Zentner, and C. N. Nagy. 2006. Energy use and efficiency in two Canadian organic and conventional crop production systems. Renewable Agriculture and Food Systems 21: 60-67.
-Hühn, M. 1996. Nonparametric analysis of genotype x environment interactions by ranks. p. 235–271. In M.S. Kang and H.G. Gauch, Jr (ed.) Genotype by environment interaction. CRC Press, Boca
-Hussein, M. A., A. Bjornstad, and A. H. Aastveit. 2000. SASG x ESTAB: A SAS program for computing genotype x environment stability statistics. Agron. J. 92: 454–459.
-Huyghe, C. 2003. Les fourrages et la production de proteins. In Actes des Journees AFPF, 17-32. AFPF, Paris.
-IFOAM. 2002. Basic standards for organic production and processing. Available at: www.ifoam.org. International Federation of Organic Agriculture Movements, Tholey-Theley, Germany.
-Irwin, J. A. G., D. L. Lloyd, and K. F. Lowe. 2001. Lucerne biology and genetic improvement-an analysis of past activities and future goals in Australia**.** *Aust. J. Agric. Res*, 52: 699–712
-Ismail, A. M., A. E. Hall, and E. M. Bray. 1994. Drought and pot size effects on transpiration efficiency and carbon isotope discrimination of cowpea accessions and hybrids. Aust. J. Plant Physiol. 21**:** 23–35.
-Ivanov, A. I. 1977. History, origin and evolution of the genus *Medicago*, subgenus Falcago. Bulletin Applied Genetics and Selection 59: 3–40.
-Jefferies, R. A., and D. K. L. Mackerron. 1997. Carbon isotope discrimination in irrigated and droughted potato (*Solanum tuberosum* L.). Plant, Cell and Environment, 20: 124-130.
-Jessen, D. L., D. K. Barnes, and C. P. Vance. 1988. Bidirectional selection in alfalfa for activity of nodule nitrogen and carbon assimilating enzymes. Crop Sci. 28: 18-22.
-Johnson, D. A. 1980. Improvement of perennial herbaceous plants for drought-stressed western rangelands. In: N. C. Turner and P. J. Kramer (Eds), Adaptation of plants to water and high temperatures stress. p. 419-433.
-Johnson D. A. and M. D. Rumbaugh. 1995. Genetic variation and inheritance characteristics for carbon isotope discrimination in alfalfa. Journal of range management 48 (2): 126-131.
-Johnson, L. D., J. J. Marquez-Ortiz, J. F. S. Lamb, and D.K. Barnes. 1998. Root morphology of alfalfa plant introductions and cultivars. Crop Sci. 38: 497–502.
-Johnson. R. C. and L .L. Tieszen. 1994. Variation for water-use efficiency in alfalfa germplasm. Crop Sci. 34: 452-458.

-Jones, H. G. 2007. Monitoring plant and soil water status: established and novel methods revisited and their relevance to studies of drought tolerance. Journal of Experimental Botany, 58 (2): 119-130.
-Kang, M. S. 1988. A rank-sum method for selecting high-yielding, stable corn genotypes. Cereal Res. Common. 16:113–115.
-Kang, M. S. 1998. Using genotype by environment interaction for crop cultivar development. Adv. Agron. 62: 199–252.
-Kang, M. S., and H. G. Gauch. 1996. Genotype -by- environment interaction. 416 pages, CRC press, Inc.
-Kang, M. S., J. D. Miller, and P. Y. P. Tai. 1983. Genetic and phenotypic path analyses and heritability in sugarcane. Crop Science. 23: 643-647.
-Katepa-Mupondwa, F. M., B. R. Christie, and T. E. Michaels. 2002. An improved breeding strategy for autotetraploid alfalfa (*Medicago sativa* L.). Euphytica 123**:** 139–146.
-Kephart, K. D., E. K. Twidwell, R. Bortnem and A. Boe**.** 1992. Alfalfa Yield Component Responses to Seeding Rate Several Years after Establishment. Agron. J. 84: 827-831
-Ketata, H., S. K. Yan, and M. Nachit. 1989. Relative consistency performance across environments. Int. Symp. on Physiology and Breeding of Winter Cereals for stressed Mediterranean Environments. Montpellier, July 3–6, 1989.
-Kozlov, N. N., M. A. Makarenkov, and T. N. Komkova. 1994. Effects and interrelations between characters of alfalfa influenced upon the content of crude protein. Report of the 34th NAAIC, Ontario, USA: 129.
-Lammerts van Bueren, E. T. 2006. Organic plant breeding: a challenge for practice and science. Paper presented at Joint Organic Congress, Odense, Denmark, May 30-31. Available at: www.louisbolk.org/downloads/1801.pdf.
-Lammerts van Bueren, E. T., P. C. Struik, and E. Jacobsen. 2002. Ecological concepts in organic farming and their consequences for an organic ideotype. Neth. J. Agric. Sci. 50:1–26.
-Lammerts van Bueren, E. T., P. C. Struik, M. Tiemens-Hulscher, and E. Jacobsen. 2003. Concepts of intrinsic value and integrity of plants in organic plant breeding and propagation. Crop Sci. 43:1922–1929
-Lammerts van Bueren, E. T., M. Hulscher, M. Haring, J. Jongerden, J. D. Van Mansvelt, A. P.M. den Nijs, and G .T. P. Ruivenkamp. 1999. Sustainable organic plant breeding. Final report: a vision, choices, consequences and steps. Louis Bolk Instituut, Netherlands.
-Lesins, K., and I. Lesins. 1979. Genus Medicago (Leguminasae). A taxogenetic study. The hague. Netherlands, Junk.

-Li, W. Z., H. Yan, Y. M. Wei, X. J. Lan, and Y. L. Zheng. 2006. Evaluation of genotype x environment Interactions in Chinese Spring Wheat by the AMMI Model, Correlation and Path Analysis. J. Agronomy & Crop Science 192: 221—227.
-Lin, C.S., and M. R. Binns. 1988. A superiority measure of cultivar performance for cultivar x location data. Can. J. Plant Sci. 68: 193–198.
-Lin, C.S., M.R. Binns, and L.P. Lefkovitch. 1986. Stability analysis: Where do we stand? Crop Sci. 26: 894–900.
-Loges, R., K. Ingwersen., A. Kaske, and F. Taube. 2000. Methodological aspects of determining nitrogen fixation of different forage legumes. Proceeding of 13th international IFOAM scientific conference, Zürich, pp 92.
-Lotti, C., P. Resta, S. Pavan, C. F. Marzano, A. R. Marcotrigiano, V. Zonno, and L. Ricciardi. 2008. Usefulness of genetic resourcesin organic farming: Case studies relative to the 'SIAB' project. Agronomiski Glasnik 4: 355-364.
-Lu, X. 1992. Comprehensive evaluation of lucerne agronomical characters with analytic hierarchy process method. *In*: Ren, J. Z. *ed.* Proceedings of the International Conference on Farming System on the Loess Plateau of China. Lanzhou , Gansu Science and Technology Press. 221– 226.
-Lu'quez, J. E., L. A. N. Aguirreza´ bal, M. E. Agu¨ ero, and V. R. Pereyra. 2002. Stability and adaptability of cultivars in non-balanced yield trials: Comparison of methods for selecting 'high oleic' sunflower hybrids for grain yield and quality. J. Agron. Crop Sci. 188: 225-234.
-Lucey, R. F., and M. B. Tesar. 1965. Frequency and rate of irrigation as factors in forage growth and water absorption, Agron. J. 57: 519-523.
-Magari, R., and M. S. Kang. 1993. Genotype selection via a new yield stability statistic in maize yield trials. Euphytica 70: 105–111.
-Marino, D., P. Frendo, R. Ladrera, A. Zabalza, A. Puppo, C. Arrese-Igor, and E. M. Gonza´lez. 2007. Nitrogen fixation control under drought stress. localized or systemic? Plant Physiology, 143: 1968–1974
-Martin, B., C. G. Tauer, and R. K. Lin.1999. Carbon isotope discrimination as a tool to improve water-use efficiency in tomato. Crop Sci. 39: 1775–1783.
-Mederksi, H. J. and D. L. Jeffers. 1973. Yield response of soybean varieties grown at two soil moisture stress levels. Agron. J. 65: 410-412.
-Melton, B., M. Wilson, and C. Currier. 1989. Registration of 'Wilson' alfalfa. Crop Sci. 29: 485-486.
-Michaud, R., W. F. Lehman, and M. D. Rumbaugh. 1988. World distribution and historical development, p. 25-91. In: A. A. Hanson, D. K. Barens, and R. R. Hill, Jr. (ed.), Alfalfa and alfalfa improvement, No. 29 in Agronomy Series. Amer. Soc. Agron. Madison, Wisc. USA.

-Montanez, A. 2000. Overview and case studies on biological nitrogen fixation: perspectives and limitations. FAO. Available at http://www.fao.org/landandwater/agll/soilbiod/caselist.stm.
-Nassar, R., and M. Hühn. 1987. Studies on estimation of phenotypic stability: Tests of significance for nonparametric measures of phenotypic stability. Biometrics 43: 45–53.
-Newbould, P. 1989. The use of nitrogen fertilizer in agriculture. Where do we go practically and ecologically?, Plant Soil 115: 297–311.
-Nguyen, H. T. 1999. Molecular dissection of drought resistance in crop plants: from traits to genes. In J. M. Ribaut and D. Poland (Eds.), Molecular Approaches for the Genetic Improvement of Cereals for Stable Production in Water-Limited Environments. Final report of workshop, 21-25 June, CIMMYT, Mexico.
-Nutman, P. S. 1967. Varietal differences in the nodulation of subterranean clover. Aust. J. Agric. Res. 18: 381-425.
-Pazdernik, D. L., P. H. Graham, C. P. Vance, and J. H. Orf. 1996. Host genetic variation in the early nodulation and dinitrogen fixation of soybeans. Crop Sci., 36: 1102-1107.
-Peoples, M., T. Swan, and J. Angus.2009. Nitrogen: Using legumes to grow your own. Available at http://www.riverineplains.com.au/GRDC Update 09 Mark Peoples.pdf. Access on 20.07.2009.
-Peoples, M. B., A.W. Faizah, , B. Rerkasem, , and D.F. Herridge. 1989a. Methods for evaluating nitrogen fixation by legumes in the field. Australian center for international agriculture research.
-Peterson, P.R., C. C. Sheaffer, and M. H. Hall.1992. Drought effects on perennial forage legume yield and quality. Agronomy Journal, 84: 774-779.
-Piepho, H. P. 2009. Available at: https://www.uni-hohenheim.de/bioinformatik/beratung /toolsmacros/sasmacros/mult.sas. Access on 02.03.2009.
-Pietola, L. and L. Alakukku. 2005. Root growth dynamics and biomass input by Nordic annual field crops. Agriculture, Ecosystems and Environment. doi:10.1016/j.agee.
-Pietsch, G. 2004. N_2-Fixierungsleistung und Wasserverbrauch von Futterleguminosen im Ökologischen Landbau unter den klimatischen Bedingungen der pannonischen Region Österreichs. Dissertation Universität für Bodenkultur, Wien.
-Pietsch, G., J. K. Friedel, and B. Freyer. 2007. Lucerne management in an organic farming system under dry site conditions. Field Crops Research 102: 104–118.
-Pietsch, G., J. K. Friedel, W. Starz, S. Kikuta, W. Loiskandel, A. Strauss-Sieberth, and B. Freyer. 2006. Biological nitrogen fixation of different legume species under water stress. Final report of BIOfix project, component B-field trial, University of natural resources and applied life sciences,Vienna.
-Popovic, S., T. Cupic, S. Grljusic, and M. Tucak. 2006. Use of variability and path analysis in determining yield and quality of alfalfa. In D. Rosellini and F. Veronesi (Eds.), Breeding and seed

production for conventional and organic agriculture. Proceeding of XXVI meeting of the EUCARPIA fodder crops and amenity grasses section, XVI meeting of the EUCARPIA *Medicago* spp group, 3-7 September, Perugia, Italy.

-Posselt, U. K. 1994. Selection parameters of quality traits in perennial ryegrass. Proceeding of Eucarpia Fodder Crops Section.129–135.

-Purchase, J. L. 1997. Parametric stability to describe GE interactions and yield stability in winter wheat. PhD. Thesis, Department of Agronomy, Faculty of Agri., Univ. of Orange Free State, Bloemfontein, South Africa.

-Quiros, C. F., and G. R. Bauchan. 1988. The genus Medicago and the origin of the *Medicago sativa* complex. In: A.A. Hanson, D.K. Barnes, and R.R. Hill (Eds.), Alfalfa and alfalfa improvement, Agronomy 29: 93–121.

-Raeini-Sarjaz, M., N. N. Barthakur, N. P. Arnold, and P. J. H. Jones. 1997. Water stress, water use efficiency, carbon isotope discrimination and leaf gas exchange relationships of bush bean. J. Agronomy and Crop Sci. 180: 173-179.

-Ray, I. M., M. S. Townsend, and J. A. Henning. 1998. Variation for yield,water use efficiency, and canopy morphology among nine alfalfa germplasms.Crop Sci. 38:1386-1390.

-Ray, I. M., M. S. Townsend, and C. K. Muncy. 1999a. Heritabilities of water-use efficiency traits and correlations with agronomic traits in water-stressed alfalfa. Crop Sci. 39:494–498.

-Ray, I. M., M. S. Townsend and C. M. Muncy.1999b. Heritabilities and interrelationships of water-use efficiency and agronomic traits in irrigated alfalfa. Crop Sci. 39: 1088–1092.

-Ray, I. M., A. Segovia-Lerma, and L. W. Murray. 2004. Diallel analysis of carbon isotope discrimination and its association with forage yield among nine historically recognized alfalfa germplasms. Crop Sci. 44: 1970-1975.

-Read, J. J, D.A. Johnson, K. H. Asay, and L .L. Tieszen. 1991. Carbon isotope discrimination, gas exchange, and water-use efficiency in crested wheatgrass clones. Crop Sci. 31: 1203–1208.

-Ren, J. 1998. Design and application of comprehensive indexing system for drought resistance evaluation of forages. Acta Pratacultural Sinica 7 (3): 34–40.

-Rennie, R. J., and G. A. Kemp. 1983. Nitrogen fixation in field beans quantified by ^{15}N isotope dilution. 2. Effect of cultivars of beans. Agron. J. 75: 645-649.

-Richards, R. A. 1996. Defining selection criteria to improve yield under drought. Plant Growth Regul. 20: 157–166.

-Riday, H., E. C. Brummer, T. A. Campbell, P. Luth, and P. M. Cazcarro. 2003. Comparisons of genetic and morphological distance with heterosis between *Medicago sativa* subsp. Sativa and subsp. Falcate. Euphytica, 131: 37-45.

-Robinson, P. H., D. H. Putnam and E. J. DePeters. 2007. Fundamentale of alfalfa quality. In: Proceedings, 37th California Alfalfa and Forage Symposium, Monterey, CA, 17-19 December, available at: http://alfalfa.ucdavis.edu.

-Rosielle, A. A., and J. Hamblin. 1981. Theoretical aspects of selection for yield in stress and non-stress environment. Crop Sci. 21: 943–946.

-Rotili, P., G. Gnocchi, C. Scotti, and D. Kertikova. 2001. Breeding of the alfalfa plant morphology for quality. Quality in Lucerne and Medics for Animal prod., Proc. Of the XIV EUCARPIA *Medicago* spp. Group Meeting, Instituto Agronomico Mediterraneo de Zaragoza: 25-57.

-Rotili, P., P. Valentini, N. Berardo, and L. Zannone. 1992. Stratigraphical analysis of experimental Lucerne meadow. In: P. Rotili and L. Zannone (Eds.), Proceeding of the Eucerpia X *Medicago sativa* Group Meeting, The future of Lucerne: Biotechnology, breeding and variety constitution, pp. 466-473. ISCF Lodi, Italy.

-Rotili, P., C. Scotti, L. Zannone, and G. Gnocchi. 1994. Lucerne stand system: dynamics of forage production, quality and demography; consequences on the variety constitution process. REUR Technical Series, FAO, 36: 54-62.

-Roy, N. N. and O. R. Murty. 1970. A selection procedure in wheat for stress environment. Euphytica 19: 509-521.

-Salter, M., B. Melton, M. Wilson, and C. Currier. 1984. Selection in alfalfa for forage yield with three moisture levels in drought boxes. Crop Sci. 24: 345–349

-SAS. 2004. SAS/STAT user guide version 9.1, SAS Institute Inc., Cary, NC, USA.

-Scheiner, S. M., R. J. Mitcell, and H. S. Callahan. 2000. Using path analysis to measures natural selection. J. Evol. Biol. 13: 423-433.

-Schowalter, T. D. 2006. Insect ecology: An ecosystem approach. 2nd Edition. Academic Press.

-Scotti, C., G. Gnocchi, M. Carelli, B. Pintus, A. Ursino, and M. Odoardi. 2006. Breeding of the alfalfa stem morphology for quality. In D. Rosellini and F. Veronesi (Eds.), Breeding and seed production for conventional and organic agriculture. Proceeding of XXVI meeting of the EUCARPIA fodder crops and amenity grasses section, XVI meeting of the EUCARPIA *Medicago* spp group, 3-7 September, Perugia, Italy.

-Sheaffer, C. C., C. B. Tanner, and M. B. Kirkham. 1988. Alfalfa water relations and irrigation, p. 373-409. In: A. A. Hanson, D. K. Barnes, and R. R. Hill, Jr. (ed.), Alfalfa and alfalfa improvement, No. 29 in Agron. Series. Amer. Sot. Agron., Madison, Wise.

-Showemimo, F. A and D. Olarewaju. 2007. Drought tolerance indices in sweet pepper *(Capsicum annuum* L.). International Journal of Plant Breeding and Genetics 1: 29-33

-Shukla, G. K. 1972a. Some aspects of partitioning genotype-environthe mental components of variability. Heredity 28: 237–245.

-Sio-Se Mardeh, A., A. Ahmadi, K. Poustini, and V. Mohammadi. 2006. Evaluation of drought resistance indices under various environmental conditions. Field Crops Research 98: 222–229.
-Skinner, D. Z., T. Loughin, and D. E. Obert. 2000. Segregation and conditional probability association of molecular markers with traits in autotetraploid alfalfa. Molecular Breeding, 6: 295-306.
-Tarakanovas, P. and A. Sprainaitis. 2005. Main additive effect and multiplicative interaction analysis of white clover genetic resources. BIOLOGIJA. 4: 38–42.
-Teulat, B., O. Merah, and D. This. 2001. Carbon isotope discrimination and productivity in field grown barley genotypes. J. Agron. Crop Sci. 187: 33—39.
-Tilman, D. 1999. Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. Proc. Nat. Acad. Sci. USA 96: 5995-6000.
-Torricelli, R. 2006. Evaluation of Lucerne varieties for organic agriculture. In D. Rosellini and F. Veronesi (Eds.), Breeding and seed production for conventional and organic agriculture. Proceeding of 26th meeting of the EUCARPIA fodder crops and amenity grasses section, 16th meeting of the EUCARPIA *Medicago* spp group, 3-7 September, Perugia, Italy.
-Tucak, M., S. Popovic, T. Cupic, S. Grljucic, S. Bolaric, and V. Kozumplik. 2008. Genetic diversity of alfalfa (*Medicago* spp.) estimated by molecular markers and morphological characters. Periodicum biologorum 110 (3): 243–249.
-van Eeuwijk, F. A. 1995. Linear and bilinear models for the analysis of multi-environment trials: I. An inventory of models. Euphytica 84: 1-7.
-van Oosterom, E. J., M. L. Whitaker, and E. Weltzien. 1996. Integrating genotype by environment interaction analysis, characterization of drought patterns, and farmer preferences to identify adaptive plant traits for pearl millet. In: Cooper, M., Hammer, G.L. (Eds.), Plant Adaptation and Crop Improvement. CAB International, Wallingford, UK, pp. 383–402.
-Vance, C. P. 2001. Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. Plant Physiol. 127: 390-397.
-Veronesi, F., C. Huyghe, and I. Delgado. 2006. Lucerne breeding in Europe: results and research for future development. Proceeding of 21th general meeting of grassland federation, Badjaz, Spain, 3-6 April.
-Willer, H., M. Yussefi-Menzler, and N. Sorensen. 2008. The World of organic agriculture statistics and emerging trends. International Federation of Organic Agriculture Movements (IFOAM) Bonn, Germany and Research Institute of Organic Agriculture (FiBL), Frick, Switzerland. Avaialable at http://www.soel.de/fachthemen/oekolandbau_welt.html.

-Wilson, M., B. Melton, J. Arledge, O. Ba1tensperger, R. J. Salter, and C. Edminster. 1983. Performance of alfalfa cu1tivars under less than optimum conditions. New Mexico Agric. Exp. Stn. Bull. No. 702.

-Xu, B., L. Shan, F. Li, and J. Jiang. 2007. Seasonal and spatial root biomass and water use efficiency of four forage legumes in semiarid northwest China. African Journal of Biotechnology 6 (23): 2708-2714.

-Yan, W., and L. A. Hunt. 1998. Genotype by environment interaction and crop yield. Plant breeding review, Vol. 16. John Wiley and Sons, Inc.

-Yan, W., M. S. Kang, B. Ma, S. Woods, and P. L. Cornelius. 2007. GGE biplot vs. AMMI analysis of genotype-by-environment data. Crop Sci. 47: 643-655.

-Zahran, H. H. 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol. Mol. Biol. Rev. 63: 968–989

-Zhao, B., M. Kondo, M. Maeda, Y. Ozaki, and J. Zhang. 2004. Water-use efficiency and carbon isotope discrimination in two cultivars of upland rice during different developmental stages under three water regimes. Plant and Soil 261**:** 61–75.

-Zobel, R. W. 1988. Statistical analysis of a yield trial. Agron. J. 80: 388-393.

-Zobel, R. W. 1992b. Soil environment constraints to root growth. Advances in soil science. 19: 27-51.

-Zobel, R. W. 1994. Stress resistance and root systems. In proceeding of the workshop on adaptation of plant to soil stresses, August 1-4, 1993, Nebraska, pp 80-99.

-Zobel, R. W., M. J. Wright, and H. G. Gauch. 1988. Statistical analysis of a yield trial. Agron. J. 80: 388-393.

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