Biological nitrogen fixation and growth parameters correlations of alfalfa (*Medicago sativa* L.) genotypes under organically managed fields with limited irrigation

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Abstract

To identify the effective characters and their relative importance in improvement of BNF, two separate field experiments were conducted under irrigated and rain-fed organic managements of dry, Pannonian region of east Austria. The experiments were laid out in an α-lattice design with two replications and 18 genotypes (eight Iranian ecotypes and ten European cultivars). Plant height was positively and significantly correlated with leaf area index (LAI) and shoot dry matter (DM) under both conditions. Positive correlations were found between biological nitrogen fixation (BNF) and shoot DM (r = 0.61** and 0.87**, irrigated and rain-fed management, respectively). Regarding correlation coefficients, 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 the indirect effects were negative. These can be regarded in selection models to avoid undesirable negative effects. Plant height and LAI can be considered as primary selection criteria for improving shoot DM, while crop re-growth and plant height, with antonymous effects, were more important for improving root dry matter.

Introduction

Legume fodder crops such as alfalfa are an essential component of organic system especially in arid and semiarid conditions. Identification of effective characters, their interrelationships and relative importance in improvement of target characters such as dry matter yield or biological nitrogen fixation (BNF) is the key step and essential part of any breeding program, especially for organic farming. BNF can be regarded as one of the most important properties of alfalfa cultivars for organic systems because it determines the N benefit to following non-legumes and thus their yield potential. Main and target traits in legume fodder crop improvement such as dry matter yield, abiotic and biotic stress tolerance, and protein content can be considered as different characters associated to the quantity of N_2 fixation. In this study, interrelationships among different agro-economic traits of 18 alfalfa genotypes from different geographical origins and also direct and indirect effects of characters on BNF, shoot and root dry matter were studied under irrigated and rain-fed organic managements in the dry, Pannonian region of east Austria.

Material and methods

Eight Iranian ecotypes and ten European cultivars were evaluated in two different conditions, irrigated in Gross-Enzersdof (48º12' N, 16º33' E) and rain-fed in Raasdorf (48º15' N, 16º37' E), of the research station of the University of Natural Resources and Life Sciences (BOKU), Vienna, Austria during 2006-08. Field experiments were established in certified organic (Austria Bio Garantie) farms where were stockless without organic manures application. The soil type in both sites was a Calcaric Phaeozem from loess with a silty loam texture. Additional information about sites and experiments is described in Moghaddam et al. (2011). The seeding density was 25 kg ha^{-1}, adjusted by the germination rate of the cultivars. The field plots, in both experiments, were laid out in an α-lattice design with two replications. Twelve characters including crop re-growth (cm) (CR), plant height (cm) (PH), number of stems per m^2 (STN), leaf to stem ratio (LSR), leaf area index (LAI), shoot dry matter (DM), stubble DM, root DM, shoot protein content (SHCP), stubble protein content (STCP), root protein content (ROCP), total biomass yield (TBS) and biological nitrogen fixation

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(BNF) were measured. Crop re-growth was measured 18-20 days after each harvest based on the average of plant height (cm) in 3 points per plot. Leaf area index (LAI) was measured using LAI-2000 Plant Canopy Analyzer (LI-COR, Lincoln, NE), before each harvest. Biological Nitrogen Fixation (BNF) was estimated by the "extended difference method" for each plot (Giller, 2001). 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. 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. Phenotypic correlations between traits based on adjusted LS-mean of genotypes across years (n=18) were calculated for each management. 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. 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.

Results

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. In this study, the correlations between some characters were condition-specific. It showed that genotypes with rapid crop re-growth in stress condition may avoid from stress effects and finally can produce more dry matter yield. Low and non-significant correlations between stem numbers per m^2 and shoots DM under both conditions can be interpreted in the way that in low input 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. Stem number is an important yield component and main trait in indirect selection for yield improvement in conventional breeding programs (Portablia et al., 1982). The positive correlation between root dry matter and crop re-growth in both conditions and negative correlation between root DM and shoot DM can explained the reason of rapid re-growth of some genotypes under irrigated condition. Specifically adapted cultivars to non-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 re-growth (Ardakani et al., 2009a,b; Annicchiarico, 2007; Avice et al., 1997; Raza et al., 2013Rotili et al., 1994; Haliloglu and Sengul, 2008). Regarding to correlations among plant height, LAI, Shoot DM and total biomass yield, it may be concluded that the high yielding genotypes had higher plants and denser stands, especially under rain-fed condition. In this study, all direct effects of BNF components were positive in both conditions, while some of indirect effects were negative. Different negative indirect effects of these main characters on BNF via each others or other characters in the model impede the BNF improvement. With regard to results of path analysis and positive cooperation in both conditions, 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 that the high yielding genotypes had higher plants and denser stands, especially under rain-fed condition. Crop re-growth 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 re-growing individuals and genotypes.

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