Genome-wide association study revealed loci linked to post-drought recovery and traits related to persistence of smooth brome grass (*Bromus inermis*)

F. Saeidnia*, M. M. Majidi, and A. Mirlohi

*Fateme Saeidnia (Corresponding author)

Ph.D of Plant Breeding, Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, Isfahan, 84156-83111, Iran

Email: f.saeidnia@alumni.iut.ac.ir

ORCID Identifier: 0000-0001-7206-8651

Mohammad Mahdi Majidi

Professor of Genetics and Plant Breeding, Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, Isfahan, 84156-83111, Iran

Email: majidi@iut.ac.ir

ORCID Identifier: 0000-0003-4746-9036

Aghafakhr Mirlohi

Professor of Genetics and Plant Breeding, Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, Isfahan, 84156-83111, Iran

Email: mirlohi@iut.ac.ir

ORCID Identifier: 0000-0002-3445-5770

Running Head: GWAS in smooth brome grass

Date of submission: 16 May 2021

Number of Tables: 2

Number of figures: 2

Word count: 2958 words

Highlight

In this study, stable marker-trait associations (MTAs) between years and moisture regimes (normal and water stress environments) were identified in a diverse panel of polycross derived progenies of smooth brome grass.

57 **Abstract**

58 Association analysis has been proved as a powerful tool for genetic dissection of complex traits. This study
 59 was conducted to identify marker–trait associations for recovery, persistence, and as well as finding stable
 60 associations. In this study, a diverse panel of polycross derived progenies of smooth brome grass was
 61 phenotyped under normal and water stress, during three consecutive years. Association analysis was performed
 62 between nine important agronomic traits along with three seasonal growth activity indices based on 535 SRAP
 63 markers. Population structure analysis identified five main subpopulations possessing significant genetic
 64 differences. Association analysis using mixed linear model identified 339 and 233 marker-trait associations
 65 under normal and water stress environments, respectively. Some of these markers were associated with more
 66 than one trait; which can be attributed to pleiotropic effects or to a number of tightly linked genes affecting
 67 several traits. If the effectiveness of these markers in genetic control of these traits is validated, they could be
 68 potentially used for initiation of marker-assisted selection and targeted trait introgression of smooth
 69 brome grass under normal and water stress environments.

70

71 **Keywords:** GWAS- linkage disequilibrium- Population structure- Post-drought recovery- Persistence- Smooth
 72 brome grass.

73

74 **Abbreviations**

75 DMY1, dry matter yield of cut 1; DMY2, dry matter yield of cut 2; DRAD, degree of recovery after drought;
 76 GCV, genotypic coefficient of variation; GLM, general linear model; GWAS, genome wide association study;
 77 LD, linkage disequilibrium; MAS, marker assisted selection; MLM, mixed linear model; MTA, marker trait
 78 association; PCV, phenotypic coefficient of variation; PER, persistence; QTL, quantitative trait loci; RY,
 79 recovery yield; SDI, summer dormancy index; SRAP, sequence related amplified polymorphism.

80

81

Introduction

Under the climatic changing context, drought is becoming most significant and acute problem affecting growth, survival and persistence of crops in many regions of the world, particularly in arid and semi-arid regions (Mollasadeghi *et al.*, 2011; Hussain *et al.*, 2012). Developing drought-tolerant varieties is an important objective of plant breeding programs and is expected to be a key component in strategies to mitigate climate change and minimize losses due to stresses and ensure production stability (Gustafson, 2011; Öztürk *et al.*, 2014). Given the advantages that perennial forage grasses such as smooth brome grass have in the arenas of both agricultural and forage sustainability for animal farming, they can be a valuable substitute for annuals under drought conditions. In these species, forage yield, persistence and recovery are of the most important agronomic features in areas with periodic drought in summer (Annicchiarico *et al.*, 2011; Pecetti *et al.*, 2011).

Successful adoption of perennial forage species depends on their ability to both survive through repeated summer droughts as well as maintain forage productivity (Annicchiarico *et al.*, 2011; Volaire, 2008). In other words, persistence of perennial grasses is dependent on the ability of the plant to maintain a viable crown at the soil surface from which growth regenerates. When genotypes are spaced planted, the difference in performance throughout the years can give an idea of persistence (Saeidnia *et al.*, 2017b). However, it is also known that poor regrowth can result in low persistence (Nie *et al.*, 2008). Therefore, during drought, trait selection for high recovering ability may be of more economic significance than selecting for improved growth (Hung and Wang, 2005; Chai *et al.*, 2010). This will enable forage plants to persist in swards or pastures and to improve their competition with less drought-tolerant species (Kanapeckas *et al.*, 2008; Volaire *et al.*, 2014). The potential of a plant to recommence growth and grain yield after experiencing water stress is defined as drought recovery (Fang and Xiong, 2015). Therefore, successful post-drought recovery is contingent on a variety of mechanisms, such as compensatory growth of remaining tissues, retention of intact growing points throughout water stress, and mobilization of the organism's carbohydrate reserves (Chai *et al.*, 2010), and is the best criterion for drought tolerance along with the persistence.

Besides, plant survival and recovery have been associated with summer dormancy, dehydration tolerance in surviving tissues, extensive root system and the ability of roots to extract water at low soil water potentials (Norton *et al.*, 2006; Shaimi *et al.*, 2009). Summer dormancy is an endogenously and temporary suspension of visible growth of any plant structure containing a meristem (Norton *et al.*, 2006), which leads to the reduction or cessation of leaf growth and possible senescence of herbage expressed under non-limiting irrigation in summer (Norton *et al.*, 2008). Shaimi *et al.*, (2009) showed that, in orchardgrass, summer dormancy is associated with superior persistence and recovery after severe drought under arid and semi-arid conditions.

Drought tolerance is a complex quantitative trait, involving diverse and multiple molecular and physiological mechanisms, signal transduction and metabolic pathways (Shinozaki and Yamaguchi-Shinozaki, 2007). Therefore, a promising strategy to facilitate selection and breeding for drought tolerance is to identify simply inherited genetic markers linked to the traits related to drought tolerance such as drought survival, post-drought recovery, and persistence. The basic prerequisite for marker-assisted selection (MAS) is the availability of markers that are strictly associated to genes or QTLs which can be used to dissect complex traits (Ebrahimi *et al.*, 2017; Kempf *et al.*, 2017).

The first step of MAS, as an important tool for accelerating the rate of genetic gain, is to dissect marker-trait associations (MTAs) (Moose and Mumm, 2008). Genome-wide association studies (GWAS) or association mapping, which is also known as linkage disequilibrium (LD) mapping, have recently been proved to be useful and powerful alternative to bi-parental QTL mapping for identifying MTA in plant populations (Thomson, 2014; Patel *et al.*, 2015). Compared to linkage mapping, GWAS offers higher mapping resolution, is less time consuming, requires fewer resources, and evaluates a much larger gene pool rapidly (Zhu *et al.*, 2008; Korte and Farlow, 2013). The power of association analysis to identify and characterize loci associated with complex traits is highly affected by admixtures of populations (Zhang *et al.*, 2012). Therefore, in order to avoid identifying false positive or spurious associations between markers and traits, it is necessary to evaluate the population structure (Pritchard *et al.*, 2000). In addition, utilizing a mixed-model approach involving multiple levels of relatedness simultaneously, has an important role in avoidance of both types of error (types I

and II) (Yu *et al.*, 2006; Ebrahimi *et al.*, 2017). Association analysis can be performed by using general linear model (GLM) and mixed linear model (MLM). In MLM, both the kinship matrix (K) and population structure (Q) are incorporated, whereas in the GLM, only population structure information is used as a covariate (Ebrahimi *et al.*, 2017).

In recent years, association analysis in forage grasses is applied extensively for dissecting the genetic bases of different quantitative traits in several species such as perennial ryegrass (*Lolium perenne*) (Auzanneau *et al.*, 2011; Yu *et al.*, 2011; Tang *et al.*, 2013; Yu *et al.*, 2013), tall fescue (*Festuca arundinacea*) (Lou *et al.*, 2015; Sun *et al.*, 2015), and orchardgrass (*Dactylis glomerata* L.) (Yan *et al.*, 2016; Zhao *et al.*, 2017; Abtahi *et al.*, 2018b). These studies have demonstrated that GWAS is an efficient technique for identifying genomic regions linked to the quantitative traits. However, in smooth brome grass the use of association analysis in identifying links between genes or markers with complex traits such as those related to persistence and recovery after drought is still in its infancy. To the best of our knowledge, this study is the first report in this regard. Therefore, this was conducted to: i) identify genetic loci associated with the productivity, persistence, post-drought recovery, and summer dormancy under normal and water stress environments; and ii) discover stable marker loci linked to the stated traits, between moisture environments and years.

Materials and Methods

Plant materials and field evaluations

Genetic materials used in this study consisted of 216 clones randomly selected from a large nursery comprised of 1800 single spaced-plant polycrossed progenies resulting from 25 preliminary parental ecotypes of smooth brome grass (*Bromus inermis*). The 25 parental genotypes of the polycross population were collected from different regions (mainly Iran) and established in the field (Supplementary Table S1). Polycross seeds from the 25 parents were grown in plastic boxes in a greenhouse during the winter of 2010. Established seedlings were space planted in the field in first March 2010 and grown during 2011 and 2012. For the present study, 216 clones were randomly selected from the nursery, propagated in a greenhouse during winter 2012 and

transferred to another field experiment, according to a randomized complete block design with 12 replications (Six replications for each normal and water stress environments) in March 2012. Spaced plants in each block were grown with 50-cm spacing between and within the rows.

Genotypes were evaluated under normal and water stress environments during 2013-2016, in which irrigation was occurred when 50% and 85% of the total available soil water was depleted from the root zone, respectively, following accepted methods of determination of evapotranspiration (Allen *et al.*, 1998). Water stress was continuously applied in each year of the experiment from early May to early October (plants growing period). In this period, depending on the weather conditions, the irrigation intervals were variable during the growing season and between the two moisture environments. To determine the gravimetric soil-water content and detect the irrigation times, soil samples were daily taken from different sites of each moisture environment before irrigation at depths of 0–20, 20–40, and 40–60 cm, using a hand auger (Clarke Topp *et al.*, 2008). The irrigation depth was calculated by the following formula:

$$I = [(FC - G_{irr}) / 100] D \times B$$

where I is the irrigation depth (cm), FC is the soil gravimetric moisture percentage at the field capacity, G_{irr} is the soil gravimetric moisture percentage at the time of irrigation, D is the root-zone depth, and B is the soil bulk density at root-zone (1.4 g cm^{-3}). A basin irrigation system was used for watering. In this system, water was delivered to the field via a pump station and polyethylene pipes. A volumetric counter was used to measure water volume applied under each moisture environment.

Phenotyping

During plant establishment year no data were recorded. Traits were measured for three years during the growing seasons of 2013 to 2015. In these years, when flowering in all plots was completed (about early summer), the aboveground biomass of each plant was harvested manually from 5 cm aboveground, dried at 75°C for 48 h and then dry matter yield weight per plant was recorded. In each year of the experiment, two harvests of above-ground biomass were undertaken. The first harvest was done after pollination assessment in

late spring (spring forage yield; SPFY, DMY1), and the second in late summer (summer forage yield; SUFY, DMY2) to assess complete growth. To evaluate the seasonal growth activity, summer dormancy index (SDI) was calculated as the ratio of the SUFY of a genotype to the SPFY of the same genotype as follows (Norton *et al.*, 2008):

$$SDI = \{100 - [(summer\ forage\ yield / spring\ forage\ yield) \times 100]\} / 10$$

After three years of field evaluation, all genotypes were assessed for post-drought recovery in the field in 2016. For this purpose, after the first harvest, a severe water stress was imposed on both previous moisture environments (normal and water stress) by stopping irrigation for 60 days (from 1st June to 31st July) until complete desiccation of the grass foliage. Then to allow for water stress recovery, all plants were subsequently irrigated to the point of field capacity every week. After six weeks of regular re-watering, traits related to recovery were measured. Recovery yield (RY) was obtained by measuring the above-ground biomass of each genotype after withholding irrigation and re-watering. The degree of recovery after drought (DRAD) was visually scored based on a scale of 0 to 9. In this respect, green and fully hydrated leaves were rated as 9 and desiccated brown/dead leaves were graded as zero. Persistence (PER) of genotypes was calculated as the difference of dry matter yield of the first cut at the fourth year (2016) from dry matter yield of the first cut at second year (2014) (Saeidnia *et al.*, 2017b):

$$PER = DMY1 (2016) - DMY1 (2014)$$

Genotyping

Young leaf tissues of smooth brome grass plants was used for extraction of genomic DNA, using the modified method described by Murray and Thompson (1980). The quality and quantity of DNA were determined by electrophoresis in 1% agarose gel. Genotyping using sequence related amplified polymorphism (SRAP) markers was performed following the method of Li and Quiros (2001). Among the SRAP markers available, 30 primer combinations were screened by polymerase chain reaction (PCR). PCR reactions were conducted in a final volume of 10 μ L consisted of 1.5 μ L of DNA, 1 μ L of forward primer, 1 μ L of reverse primer, 5 μ L of

master mix (Amplicon) and 1.5 μ L of distilled water, using a BIO-RAD thermocycler. For SRAP analysis, samples were subjected to the following thermal profile: the five cycles including initial denaturation of 1 min at 94°C, annealing of 1 min at 35°C, and extension of 1 min at 72°C, followed by 35 cycles of 3 min at 50°C, with a final extension of 10 min at 72°C. PCR products were separated by electrophoresis on 12% non-denatured polyacrylamide gels and stained by AgNO₃ solution (Bassam et al., 1991). Polymorphic SRAP markers were scored as binary data with presence (1) or absence (0).

Statistical analyses

The Kolmogorov–Smirnov and Bartlett’s tests were used to examine the normality and homogeneity of variance, respectively. Analysis of variance and estimation of variance components for the normal and water stress environments separately were performed for all measured traits using PROC Mixed in SAS release 9.4 (SAS Institute, 2011). Least significant difference (LSD) test at $P \leq 0.05$ was used for trait means comparison (Steel and Torrie, 1980). Broad-sense heritability (h^2_b) was estimated for normal and water stress environments on a phenotypic mean basis averaged over replications as described by Nguyen and Sleper (1983):

$$h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_{gy}^2/y + \sigma_{gr}^2/r + \sigma_e^2/ry)$$

where σ_g^2 is the genotype, σ_{gy}^2 is the genotype $\times$ year, σ_{rg}^2 is the genotype $\times$ rep and σ_e^2 is the residual variance, y is the number of years and r is the number of replicates. To estimate the level of genetic variation, the phenotypic coefficient of variation (PCV) and genetic coefficient of variation (GCV) were calculated as:

$$PCV = (\sigma_p / \mu) 100$$

$$GCV = (\sigma_g / \mu) 100$$

where σ_p is the standard deviation of the phenotypic variance, σ_g is the standard deviation of the genotypic variance, and μ is the phenotypic mean (Falconer and Mackay, 1996).

Population structure and association analysis

Structure analysis and stratification of the studied population into subpopulations with different genetic structures was done based on SRAP marker data in STRUCTURE software version 2.3.4 (Pritchard *et al.*, 2000). This analysis was performed applying an admixture model, a burn-in of 10,000 iterations followed by 100,000 Monte Carlo Markov Chain (MCMC) replicates. The membership of each genotype was run for the range of genetic clusters (K) from K= 2 to K= 10 with five repetitions for each K. Delta k approach by Evanno *et al.* (2005) was used to determine the optimum number of sub-populations, using STRUCTURE HARVESTER (Earl and VonHoldt, 2012).

Association analysis was run by MLM (Yu *et al.*, 2006) to calculate *P*-values for marker–trait associations, using TASSEL version 4.2.1 (Bradbury *et al.*, 2007). The phenotypic mean of traits (P-matrix) was applied to identify significant associations under normal and water stress environments, separately. To correct for population structure in MLM model, the Q-matrix derived from structure analysis (at maximum DK), was used as a covariate. Moreover, a kinship matrix (K-matrix) was calculated based on the results of marker genotype data using TASSEL version 4.2.1 (Bradbury *et al.*, 2007) and was used. A correction for multiple testing was performed with the FDR (false discovery rate) method, using the QVALUE R package (Storey, 2002).

Results

Phenotyping

Mean comparisons of all measured traits for the two moisture environments are given in Table 1. Results showed that moisture environment had significant effect on all of the evaluated traits and the magnitude of mean performance was significantly decreased for all traits under water stress environment (Table 1). Under water stress, DMY1 was approximately reduced by 36, 39 and 37% during 2013, 2014, and 2015, respectively, when compared with normal environment. For DMY2, these reductions were approximately 38, 60, and 56%, in the same three consecutive years relative to normal environment.

Phenotypic coefficient of variation (PCV) was from 3.82% for SDI-Y1 to 87.24% for PER under normal environment and from 27.96% for DMY2-Y3 to 68.46% for PER under water stress environment. The range of

genetic coefficient of variation (GCV) was from 2.86% for SDI-Y1 to 68.15% for PER under normal environment and from 22.26% for DMY2-Y3 to 55.10% for RY under water stress environment. Except for DMY2-Y1, DMY1-Y3, and PER for PCV, and DMY2-Y1 and PER for GCV, the values of genetic variation under water stress were higher than the ones for normal environment (Table 1).

Broad-sense heritability estimates for combined data ranged from 50.93% for RY to 76.33% for PER. Moreover, the heritability estimates were calculated for each moisture environment, separately and are given in Table 1. Under both moisture environments, moderate to high values of heritability were estimated for all traits. According to the results, heritability estimates ranged from 52.97% for RY to 79.38% for DMY1-Y2 under normal environment and from 58.78% for PER to 78.46% for DRAD under water stress environment (Table 1).

Population structure and association analysis

The maximum likelihood and DK were used to calculate the number of subpopulations (K). The optimum number of sub-populations (K) was determined based on 30 SRAP primer combinations using the largest value of Delta K in the STRUCTURE 2.3.4 software. The maximum value of DK obtained at $K=5$, suggesting that there is five subpopulations in the smooth brome grass panel (Supplementary Table S2; Figs. 1 and 2).

Association analysis between SRAP markers and the phenotypic mean of traits was separately conducted for normal and water stress environments, based on MLM model. In this model, kinship or relatedness matrix was considered as a factor. Under normal environment (P values <0.01 and a cut-off value of 0.05 for the FDR) 267 and 72 SRAP markers showed significant associations with means of the studied traits, at 0.05 and 0.01 probability levels, respectively (Table 2). The percentage of phenotypic variation (coefficient of determination, R^2) of an individual trait explained ranged from 1.63% to 11.38% (Table 2). Under water stress 185 and 48 markers had significant association with the studied traits, at the 0.05 and 0.01 probability levels, respectively. The percentage of phenotypic variation (R^2) of a trait explained varied from 1.03% to 16.03% (Table 2). It should be stated that, from SRAP markers which were associated with studied traits under normal

environment, 72 and 22 markers were significantly associated with SDI-Y1, SDI-Y2, and SDI-Y3 (which were only calculated under normal environment), at the 0.05 and 0.01 probability levels, respectively (Table 2).

Association analysis based on MLM model showed markers which were associated with more than one trait at the same time. Under normal environment 72 markers and under water stress 61 markers showed significant associations with more than one trait, simultaneously. For example, under normal environment, marker Me4/Em2-12 showed significant associations with DMY1-Y1, DMY2-Y1, DMY1-Y2, DMY2-Y2, DMY1-Y3, DMY2-Y3, RY, and DRAD and marker Me4/Em6-20 had significant associations with DMY1-Y2, DMY2-Y2, DMY1-Y3, DMY2-Y3, and RY concurrently. In addition, under water stress situation, marker Me4/Em6-10 showed significant associations with DMY1-Y1, DMY2-Y1, DMY1-Y2, DMY2-Y2, DMY1-Y3, DMY2-Y3, RY, and DRAD and marker Me4/Em6-20 had significant associations with DMY1-Y2, DMY1-Y3, DMY2-Y3, RY, and DRAD (Table 2).

Association analysis was performed in each moisture environment separately to assess stable associations. In total, 75 trait associated markers showed sufficiently stable expression across moisture environments. For instance, markers Me5/Em4-4, Me1/Em1-17, Me4/Em6-10, Me4/Em6-20, and Me5/Em6-4 showed significant and stable associations with DMY1-Y2 in both moisture environments. Similarly, markers Me5/Em4-7, Me1/Em4-19, Me1/Em2-15, Me4/Em3-11, Me2/Em1-1, Me2/Em5-7, Me5/Em6-4, Me5/Em5-6, Me1/Em3-4, and Me1/Em3-23 showed significant and stable associations with DMY1-Y1 (Table 2). Moreover, under both moisture environments, Marker Me1/Em4-19 had significant associations with DMY1-Y1 and PER. In the same way, marker Me5/Em4-7 was associated with DMY1-Y1, DMY2-Y1, and PER under normal environment and with DMY1-Y1, DMY2-Y1, DMY1-Y2, and PER under water stress environment (Table 2).

For assessing the stability of marker-trait associations (MTAs) between years, analysis was conducted on the traits of each experimental year. Results revealed 30 and 28 stable MTAs between years, under normal and water stress environments, respectively (Table 2). For example, under normal environment marker Me1/Em5-11 showed significant associations with DMY1-Y1, DMY2-Y1, DMY1-Y2, DMY2-Y2, and DMY2-Y3. Similarly, marker Me4/Em5-19 had significant and stable associations with DMY1-Y2, DMY1-Y3, SDI-Y1,

and SDI-Y2, under normal environment. On the other hand, two markers of Me2/Em1-1 and Me1/Em1-17 showed significant and stable associations with DMY1-Y1, DMY2-Y1, DMY1-Y2, and DMY1-Y3, under water stress environment.

Discussion

The quantitative inheritance of drought tolerance and interaction between gene expression and environment has challenged the understanding of genetic basis of drought tolerance-related traits in plants (Sun *et al.*, 2015). In the present study significant genetic variations were observed among genotypes in terms of all measured traits demonstrating the difference in genes controlling these traits. The non-static performance of genotypes in the two moisture environments emphasizes the importance of marker-trait association analysis in the two moisture environments, separately.

All traits were significantly affected by water stress more likely due to decreased water potential of the soil and decline in net assimilation and photosynthesis of leaves (Flexas *et al.*, 2004; Merewitz *et al.*, 2010). Saeidnia *et al.* (2017b) in smooth brome grass (*Bromus inermis*) and Majidi *et al.* (2016) in orchardgrass (*Dactylis glomerata*) reported similar results. Wide genetic variation observed for all studied traits revealed potential for genetic gain from selection in this germplasm. Moreover, higher estimates of PCV and GCV for most of the evaluated traits under water stress compared with normal environment indicates that water stress have increased genetic variation and therefore, selection under this condition would be more effective. The findings in this context are contradictory. For example, some researchers believe that genetic gain through selection is higher under normal environment than water limited conditions (Blum, 2011; Majidi *et al.*, 2016). While, others have reported higher genetic advance through selection under water stress (Abtahi *et al.*, 2018a; Saeidnia *et al.*, 2017a). Moderate to high heritability estimates observed for all of the traits emphasizes that detecting of marker-trait associations is possible for these traits (Hung *et al.*, 2012).

Population structure analysis separated the smooth brome grass genotypes into five groups with different genetic structures. Furthermore, association analysis of evaluated traits under normal and water stress

conditions revealed that MTAs were mostly different at the two environments. Moreover, results showed that a greater number of genes were involved in controlling traits under normal environment than water stress. The percentage of variation which is explained by each identified association was low, that may be due to the role of many minor genes controlling the trait, outcrossing nature of smooth brome grass, markers exhibiting minor quantitative effect, rare alleles, and complex allelic interactions (Yang *et al.*, 2010; Debibakas *et al.*, 2014). Similar results were reported by Lou *et al.* (2015) and Sun *et al.* (2015) in tall fescue.

Based on the results, some markers had simultaneously significant associations with more than one trait and may be effectively used for the improvement of several traits, concurrently (Sun *et al.*, 2015; Abtahi *et al.*, 2018b). Association between multiple traits could be attributed to the co-expression mediated by expression of quantitative trait loci or e-QTLs (House *et al.*, 2014). For instance, marker Me2/Em1-19 showed simultaneously significant associations with DMY1-Y2, DMY2-Y3, RY, DRAD, and SDI-Y1 under normal environment. Similarly, marker Me4/Em3-11 concurrently showed significant associations with DMY1-Y1, DMY2-Y1, DMY1-Y2, DMY2-Y2, and PER, under water stress environment. The simultaneous associations of markers with multiple traits have been attributed to the pleiotropic effects or to several tightly linked genes affecting the traits (Lehner, 2011; Sun *et al.*, 2015).

Improving the persistence, recovery, and survivability of perennial forage grasses is one of the main objectives of grass breeders in areas with prolonged periods of drought (Annicchiarico *et al.*, 2011; Pecetti *et al.*, 2011). In this study, markers associated with these traits were identified under normal and water stress environments. For instance, markers Me5/Em5-19, Me5/Em6-16, Me5/Em2-15, Me4/Em3-11, Me4/Em2-28, Me5/Em6-1, Me2/Em1-2, Me4/Em3-14, Me5/Em4-7, Me2/Em2-14, Me2/Em2-17, Me4/Em6-7, Me1/Em4-19, and Me1/Em3-18 were associated with superior persistence under both moisture environments. Similarly, marker Me4/Em6-20 was associated with RY, and marker Me5/Em5-9 was associated with DRAD, under normal and water stress environments. If the effectiveness of these regions in the genetic control of these traits is confirmed, these markers could be potentially used for the improvement of recovery, persistence and therefore drought tolerance of smooth brome grass. Moreover, an important trait associated with survivability,

persistence and recovery after drought is summer dormancy; which improves autumn recovery and therefore results in a better persistence of perennial grasses. In the present study 30, 29, and 35 markers were associated with SDI-Y1, SDI-Y2, and SDI-Y3, respectively. From these markers, 24 were also associated with other traits and four markers showed stable association and were associated with SDI at two or three years of study.

In the present study most of the MTAs were different for the two moisture environments and also for the three experimental years, indicating the considerable role of environmental effects in these associations (Bocianowski and SeidlerŁozykowska, 2012). These findings may suggest that different genes contribute to the same trait in different environments and years (Rumbaugh *et al.*, 1984) or the same genes may change the expression level between different environments (House *et al.*, 2014). In the present study, 75 markers showed stable association with different traits under both moisture environments. Moreover, 30 and 28 markers showed stable MTAs between three years of study, under normal and water stress environments, respectively. In general, associated markers which were detected in two or more different environments or experimental years are more reliable than those present in only one environment (Diapari *et al.*, 2015).

In conclusion, the advantage of association analysis technique as a powerful tool to identify and detect genes and markers linked to complex traits of agricultural and economic importance was demonstrated. Satisfactory levels of polymorphism and genetic diversity was observed for the studied traits in the polycrossed population. Five subpopulations were identified in smooth brome grass panel; and 339 and 233 significant MTAs were detected under normal and water stress environments, respectively. Some SRAP markers were associated with the recovery and persistence of this species. Therefore, it was demonstrated that SRAP markers can be used in the future breeding program to improve recovery after prolonged drought and persistence, and hence enhance drought tolerance of smooth brome grass. Environmental specificity of MTAs indicated that genotype $\times$ environment interactions affect association analysis; nevertheless, 75 MTAs showed significantly stable expression across normal and water stress conditions. Also, 30 and 28 MTAs showed significantly stable expression across years of study, under normal and water stress conditions, respectively. The molecular

markers identified in the present study are suggested as useful genomic resources in the future breeding programs of smooth brome grass.

Supplementary data

Supplementary data are available at *JXB* online.

Table S1. Information on parental plants of genetic materials used in this study.

Table S2. Calculated statistics to detect optimum number of subgroups (*K*) in structure analysis of smooth brome genotypes (DK method; Evanno *et al.* 2005), using the program STRUCTURE.

Acknowledgements

We would like to thank *Iran National Science Foundation (INSF)*[grant number 98025737]and Isfahan University of Technology for the award of a Postdoctoral Research Fellowship to the first author.

Author contributions

FS, MMM and AM conceived and designed the experiments; FS performed the experiments, analyzed the data and wrote the manuscript with the supervision of MMM and AM; all authors discussed the results and reviewed the manuscript.

Data availability statements

The data supporting the findings of this study are available from the corresponding author, (F. Saeidnia), upon request.

References

Abtahi M, Majidi MM, Mirlohi A. 2018a. Marker-based parental selection to improve performance of orchardgrass polycross populations under water deficit environments. *Euphytica* 214, 232. doi: [10.1007/s10681-018-2313-1](https://doi.org/10.1007/s10681-018-2313-1)

405 **Abtahi M, Majidi MM, Mirlohi A, Saeidnia F.** 2018b. Association analysis for seed yield, forage yield and
406 traits related to drought tolerance in orchardgrass (*Dactylis glomerata*). Crop and Pasture Science 69,
407 1150-1164.

408 **Allen RG, Pereira LS, Raes D, Smith M.** 1998. Crop evapotranspiration: Guidelines for computing crop
409 requirements. *FAO Irrigation and Drainage Paper* 56, 41-51.

410 **Annicchiarico P, Pecetti L, Bouzerzour H, Kallida R, Khedim A, Porqueddu C, Simões NM, Volaire F,**
411 **Lelièvre F.** 2011. Adaptation of contrasting cocksfoot plant types to agricultural environments across the
412 Mediterranean basin. *Environmental and Experimental Botany* 74, 82– 89.

413 **Auzanneau J, Huyghe C, Escobar-Gutiérrez AJ, Julier B, Gastal F, Barre P.** 2011. Association study
414 between the gibberellic acid insensitive gene and leaf length in a *Lolium perenne* L. synthetic variety.
415 *BMC Plant Biology* 11, 183. [doi:10.1186/1471-2229-11-183](https://doi.org/10.1186/1471-2229-11-183)

416 **Bassam BJ, Caetano-Anollés G, Gresshoffet PM.** 1991. Fast and sensitive silver staining of DNA in
417 polyacrylamide gels. *Analytical Biochemistry* 196, 80-83.

418 **Blum A.** 2011. Plant breeding for water limited environments. New York: Springer.

419 **Bocianowski J, Seidler-Łożykowska K.** 2012. The relationship between RAPD markers and quantitative
420 traits of caraway (*Carum carvi* L.). *Industrial Crops and Products* 36, 135-139.

421 **Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES.** 2007. TASSEL: Software
422 for association mapping of complex traits in diverse samples. *Bioinformatics* 23, 2633-2635.

423 **Chai Q, Jin F, Merewitz E, Hung B.** 2010. Growth and physiological traits associated with drought survival
424 and post-drought recovery in perennial turfgrass species. *Journal of the American Society for*
425 *Horticultural Science* 135, 125-133.

426 **Clarke Topp C, Parkin GW, Ferre TPA.** 2008. Soil water content. In: Carter MR, Gregorich EG, eds. *Soil*
427 *sampling and methods of analysis*. Pinawa: Canadian Society of Soil Science, 939-961.

428 **Debibakas S, Rocher S, Garsmeur O, Toubi L, Roques D, D'Hont A, Hoarau JY, Daugrois JH.** (2014).
429 Prospecting sugarcane resistance to sugarcane yellow leaf virus by genome-wide association. Theoretical
430 and Applied Genetics 127, 1719-1732.

431 **Diapari M, Sindhu A, Warkentin TD, Bett K, Tar'an B.** 2015. Population structure and marker-trait
432 association studies of iron, zinc and selenium concentrations in seed of field pea (*Pisum sativum* L.).
433 Molecular Breeding 35, 30. <https://doi.org/10.1007/s11032-015-0252-2>

434 **Earl DA, VonHoldt BM.** 2012. STRUCTURE HARVESTER: a website and program for visualizing
435 STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4, 359-
436 361.

437 **Ebrahimi F, Majidi MM, Arzani A, Mohammadi-Nejad G.** 2017. Association of molecular markers with
438 traits under drought stress conditions in a worldwide collection of safflower. Crop and Pasture Science 68,
439 167-175.

440 **Evanno G, Regnaut S, Goudet J.** 2005. Detecting the number of clusters of individuals using the software
441 STRUCTURE: a simulation study. Molecular Ecology 14, 2611-2620.

442 **Falconer DS, Mackay TFC.** 1996. Introduction to quantitative genetics. UK: Longman.

443 **Fang Y, Xiong L.** 2015. General mechanisms of drought response and their application in drought resistance
444 improvement in plants. Cellular and Molecular Life Sciences 72, 673–689.

445 **Flexas J, Bota J, Loreto F, Cornic G, Sharkey TD.** 2004. Diffusive and metabolic limitations to
446 photosynthesis under drought and salinity in C3 plants. Plant Biology 6, 1-11.

447 **Gustafson D. I.** 2011. Climate change: a crop protection challenge for the twenty first century. Pest
448 Management Science 67, 691–696.

449 **House MA, Griswold CK, Lukens LN.** 2014. Evidence for selection on gene expression in cultivated rice
450 (*Oryza sativa*). Molecular Biology and Evolution 31, 1514-1525.

451 **Hung HY, Browne C, Guill K, et al.** 2012. The relationship between parental genetic or phenotypic
452 divergence and progeny variation in the maize nested association mapping population. *Heredity* 108, 490–
453 499.

454 **Huang B, Wang Zh.** 2005. Physiological recovery of Kentucky bluegrass from drought stress. *International*
455 *Turfgrass Society Research Journal* 10, 867-873.

456 **Hussain SS, Raza H, Afzal I, Kayani MA.** 2012. Transgenic plants for abiotic stress tolerance: Current
457 status. *Archives of Agronomy and Soil Science* 58, 693-721.

458 **Kanapeckas J, Lemeziene N, Stukonis V, Tarakanovas P.** 2008. Drought tolerance of turfgrass genetic
459 resources. *Biologija* 54(2), 121-124.

460 **Kempf K, Malisch CS, Grieder C, Widmer F, Kölliker R.** 2017. Marker-trait association analysis for
461 agronomic and compositional traits in sainfoin (*Onobrychis viciifolia*). *Genetics and Molecular Research*
462 16(1). [doi:10.4238/gmr16019483](https://doi.org/10.4238/gmr16019483)

463 **Korte A, Farlow A.** 2013. The advantages and limitations of trait analysis with GWAS: a review. *Plant*
464 *Methods* 9 (1), 29. <https://doi.org/10.1186/1746-4811-9-29>

465 **Lehner B.** 2011. Molecular mechanisms of epistasis within and between genes. *Trends in Genetics* 27, 323-
466 331.

467 **Li G, Quiros CF.** 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a
468 simple PCR reaction: its application to mapping and gene tagging in Brassica. *Theoretical and Applied*
469 *Genetics* 103, 455–461.

470 **Lou Y, Hu L, Chen L, Sun X, Yang Y, Liu H, Xu Q.** 2015. Association analysis of simple sequence repeat
471 (SSR) markers with agronomic traits in tall fescue (*Festuca arundinacea* Schreb.). *PLoS ONE* 10(7),
472 e0133054. [doi:10.1371/journal.pone.0133054](https://doi.org/10.1371/journal.pone.0133054)

473 **Majidi MM, Hosseini B, Barati M, Mirlohi A, Araghi B.** 2016. Simultaneous selection for seed and forage
474 production in cocks-foot: application of drought tolerance and susceptibility indices. *Grass and Forage*
475 *Science* 72(3), 441-453.

476 **Merewitz E, Meyer W, Bonos S, Huang BR.** 2010. Drought stress responses and recovery of Texas x
477 Kentucky hybrids and Kentucky bluegrass genotypes in temperate climate conditions. *Agronomy Journal*
478 102, 258-268.

479 **Mollasadeghi V, Valizadeh M, Shahryari R, Imani AA.** 2011. Evaluation of end drought tolerance of 12
480 wheat genotypes by stress indices. *World Applied Sciences Journal* 13(3), 545-551.

481 **Moose SP, Mumm RH.** 2008. Molecular plant breeding as the foundation for 21st century crop improvement.
482 *Plant Physiology* 147(3), 969-977.

483 **Murray MG, Thompson WF.** 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids*
484 *Research* 8, 4321-4326.

485 **Nguyen HT, Sleper DA.** 1983. Theory and application of half-sib matings in forage breeding. *Theoretical and*
486 *Applied Genetics* 64, 187-196.

487 **Nie ZN, Miller S, Moore GA, Hackney BF, Boschma SP, Reed KFM, Mitchell M, Albertsen TO, Clark**
488 **S, Craig AD, Kearney G, Li GD, Dear BS.** 2008. Field evaluation of perennial grasses and herbs in
489 southern Australia. Persistence, root characteristics and summer activity. *Australian Journal of*
490 *Experimental Agriculture* 48, 424–435.

491 **Norton MR, Volaire F, Lelievre F.** 2006. Summer dormancy in *Festuca arundinacea* Schreb, the influence of
492 season of sowing and a simulated mid-summer storm on two contrasting cultivars. *Australian Journal of*
493 *Agricultural Research* 57, 1267–1277.

494 **Norton MR, Volaire F, Fukai S, Lelievre F.** 2008. Measurement of summer dormancy in temperate
495 perennial pasture grasses. *Australian Journal of Agricultural Research* 59, 498–509.

496 **Öztürk A, Bayram S, Haliloglu K, Aydin M, Çağlar Ö, Bulut S.** 2014. Characterization for drought
497 resistance at early stages of wheat genotypes based on survival, coleoptile length, and seedling vigor.
498 *Turkish Journal of Agriculture and Forestry* 38, 824-837.

499 **Patel D, Zander M, Dalton-Morgan J, Batley J.** 2015. Advances in plant genotyping: Where the future will
500 take us. *Methods in Molecular Biology* 1245, 1-11.

501 **Pecetti L, Annicchiarico P, Abdelguerfi A, Kallida R, Mefti M, Porqueddu C, Simões NM, Volaire F,**
502 **Lelievre F.** 2011. Response of Mediterranean tall fescue cultivars to contrasting agricultural
503 environments and implications for selection. *Journal of Agronomy and Crop Science* 197, 12-20.

504 **Pritchard JK, Stephens M, Donnelly P.** 2000. Inference of population structure using multi-locus genotype
505 data. *Genetics* 155, 945-959.

506 **Rumbaugh MD, Asay KH, Johnson DA.** 1984. Influence of drought stress on genetic variance of alfalfa and
507 wheat grass seedling. *Crop Science* 24, 297-303.

508 **Saeidnia F, Majidi MM, Mirlohi A.** 2017a. Analysis of seed production and its association with forage
509 production and agronomic traits in orchardgrass (*Dactylis glomerata*) under different moisture conditions.
510 *Crop and Pasture Science* 68, 657-669.

511 **Saeidnia F, Majidi MM, Mirlohi A, Manafi M.** 2017b. Productivity, persistence and traits related to drought
512 tolerance in smooth brome grass. *Plant Breeding* 136, 270–278.

513 **SAS Institute.** 2011. User's guide. Release 9.4. SAS Institute: Cary, NC, USA.

514 **Shaimi N, Kallida R, Volaire F, Saidi N, Faiz CA.** 2009. Summer dormancy and drought survival of
515 Moroccan ecotypes of orchardgrass. *Crop Science* 49, 1416–1424.

516 **Shinozaki K, Yamaguchi-Shinozaki K.** 2007. Gene networks involved in drought stress response and
517 tolerance. *Journal of Experimental Botany* 58, 221–227.

518 **Steel RGD, Torrie JG.** 1980. Principles and procedures of statistics. New York: McGraw–Hill Book, Co.

519 **Storey JD.** 2002. A direct approach to false discovery rates. *Journal of the Royal Statistical Society: Series B*
520 64, 479-498.

521 **Sun X, Du Z, Ren J, Amombo E, Hu T, Fu J.** 2015. Association of SSR markers with functional traits from
522 heat stress in diverse tall fescue accessions. *BMC Plant Biology* 15, 116-149.

523 **Tang JC, Camberato JJ, Yu XQ, Luo N, Bian SM, Jiang YW.** 2013. Growth response, carbohydrate and
524 ion accumulation of diverse perennial ryegrass accessions to increasing salinity. *Scientia Horticulturae*
525 154, 73-81.

526 **Thomson M.** 2014. High-throughput SNP genotyping to accelerate crop improvement. *Plant Breeding and*
527 *Biotechnology* 2(3), 195-212.

528 **Volaire F.** 2008. Plant traits and functional types to characterize drought survival of pluri-specific perennial
529 herbaceous swards in Mediterranean areas. *European Journal of Agronomy* 29, 116–124.

530 **Volaire F, Barkaoui K, Norton MR.** 2014. Designing resilient and sustainable grasslands for a drier future:
531 adaptive strategies, functional traits and biotic interactions. *European Journal of Agronomy* 52, 81– 89.

532 **Yan H, Zhang Y, Zeng B, et al.** 2016. Genetic diversity and association of EST-SSR and ScoT markers with
533 rust traits in orchardgrass (*Dactylis glomerata* L.). *Molecules* 21(1), 66. doi: [10.3390/molecules21010066](https://doi.org/10.3390/molecules21010066).

534 **Yang J, Benyamin B, McEvoy BP, et al.** 2010. Common SNPs explain a large proportion of the heritability
535 for human height. *Nature Genetics* 42, 565-569.

536 **Yu X, Bai G, Liu S.** 2013. Association of candidate genes with drought tolerance traits in diverse perennial
537 ryegrass accessions. *Journal of Experimental Botany* 64(6), 1537-1551.

538 **Yu X, Bai G, Luo N, Chen Z, Liu S, Liu J, Warnke SE, Jiang Y.** 2011. Association of simple sequence
539 repeat (SSR) markers with submergence tolerance in diverse populations of perennial ryegrass. *Plant and*
540 *Soil* 2, 391-398.

541 **Yu J, Pressoir G, Briggs WH, et al.** 2006. A unified mixed-model method for association mapping that
542 accounts for multiple levels of relatedness. *Nature Genetics* 38, 203-208.

543 **Zhang Q, Wu C, Ren F, Li Y, Zhang Ch.** 2012. Association analysis of important agronomical traits of
544 maize inbred lines with SSRs. *Australian Journal of Crop Science* 6(6), 1131–1138.

545 **Zhao X, Bushman BS, Zhang X, Robbins MD, Larson SR, Robins JG, Thomas A.** 2017. Association of
546 candidate genes with heading date in a diverse *Dactylis glomerata* population. *Plant Science* 265, 146-
547 153.

548 **Zhu CS, Gore M, Buckler ES, Yu JM.** 2008. Status and prospects of association mapping in plants. *The*
549 *Plant Genome* 1, 5–20.

Table 1- Mean performance, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) and broad- sense heritability (h^2_b) of traits recorded under normal and water stress environments in smooth brome grass genotypes.

Traits	Mean $\pm$ SD		Change (%)	PCV (%)		GCV (%)		h^2_b (%)		
	Normal	Stress		Normal	Stress	Normal	Stress	Combined	Normal	Stress
DMY1-Y1 (g/plant)	155.66 $\pm$ 41.77	100.24 $\pm$ 33.96	35.60**	26.00	33.32	22.59	29.27	73.72	75.53	77.19
DMY2-Y1 (g/plant)	30.48 $\pm$ 6.36	18.81 $\pm$ 5.42	38.29**	31.05	28.80	27.35	24.18	75.93	77.61	70.50
DMY1-Y2 (g/plant)	264.59 $\pm$ 68.05	161.33 $\pm$ 50.46	39.03**	24.81	31.16	22.11	27.48	70.20	79.38	77.81
DMY2-Y2 (g/plant)	61.76 $\pm$ 17.19	24.81 $\pm$ 7.86	59.83**	27.62	31.57	22.50	26.80	60.53	66.39	72.05
DMY1-Y3 (g/plant)	155.56 $\pm$ 42.81	98.40 $\pm$ 28.16	36.74**	27.32	28.34	22.98	24.42	72.69	70.77	74.27
DMY2-Y3 (g/plant)	36.92 $\pm$ 9.31	16.29 $\pm$ 4.55	55.88**	25.44	27.96	19.87	22.26	59.58	60.99	63.34
RY (g/plant)	39.77 $\pm$ 11.10	25.90 $\pm$ 16.75	34.87**	37.29	63.43	27.14	55.10	50.93	52.97	75.47
DRAD (0-9)	5.45 $\pm$ 1.08	3.19 $\pm$ 1.62	41.47**	19.87	50.43	14.71	44.66	54.37	54.79	78.46
PER (g/plant)	69.27 $\pm$ 49.80	49.66 $\pm$ 35.14	28.31**	87.24	68.46	68.15	52.49	76.33	61.03	58.78
SDI-Y1	8.60 $\pm$ 0.34	—	—	3.82	—	2.86	—	—	56.25	—
SDI-Y2	7.61 $\pm$ 0.56	—	—	7.30	—	5.86	—	—	64.57	—
SDI-Y3	7.44 $\pm$ 0.74	—	—	9.40	—	7.96	—	—	71.76	—

** significant at the 0.01 probability level; ns: not significant

Table 2- Association of SRAP markers with dry matter yield, persistence, summer dormancy and recovery traits of smooth brome grass under normal and water stress environments based on mixed linear model (MLM).

Trait	Normal environment			Water stress environment		
	Marker	P value	R ² (%)	Marker	P value	R ² (%)
DMY1-Y1	Me5/Em4-7 [†]	0.0005	5.44	Me2/Em4-16	0.00023	6.35
	Me1/Em4-19 [†]	0.0029	4.20	Me2/Em1-1 [†]	0.0021	4.71
	Me1/Em2-15 [†]	0.0032	4.13	Me1/Em4-19 [†]	0.0029	4.47
	Me4/Em3-11 [†]	0.0039	3.98	Me5/Em4-7 [†]	0.0038	4.25
	Me2/Em3-4	0.0112	3.16	Me4/Em1-16	0.0068	3.78
	Me2/Em2-16	0.0129	3.06	Me5/Em6-4 [†]	0.0114	3.36
	Me4/Em2-12	0.0161	2.88	Me5/Em2-13	0.0127	3.27
	Me1/Em5-11	0.0186	2.77	Me5/Em5-6 [†]	0.0127	3.27
	Me2/Em1-1 [†]	0.0193	2.74	Me5/Em4-10	0.0132	3.24
	Me5/Em1-12	0.0197	2.72	Me4/Em4-8	0.0136	3.21
	Me5/Em2-3	0.0219	2.64	Me5/Em3-23	0.0165	3.05
	Me2/Em5-7 [†]	0.0224	2.62	Me5/Em5-13	0.0179	2.98
	Me2/Em2-19	0.0237	2.57	Me1/Em2-22	0.0181	2.97
	Me1/Em3-18	0.027	2.47	Me1/Em5-23	0.0187	2.94
	Me4/Em1-17	0.0273	2.46	Me4/Em3-11 [†]	0.0206	2.86
	Me1/Em2-1	0.0279	2.44	Me4/Em6-10	0.0207	2.86
	Me5/Em6-4 [†]	0.0317	2.34	Me2/Em5-18	0.024	2.73
	Me2/Em3-3	0.032	2.33	Me2/Em2-17	0.0286	2.58
	Me4/Em3-9	0.0351	2.26	Me1/Em1-8	0.0304	2.53
	Me5/Em5-6 [†]	0.036	2.24	Me5/Em1-14	0.0312	2.51
	Me5/Em6-7	0.0367	2.22	Me2/Em5-7 [†]	0.0319	2.49
	Me2/Em4-15	0.0377	2.20	Me1/Em1-17	0.0324	2.47
	Me5/Em2-16	0.0412	2.13	Me5/Em1-15	0.035	2.41
	Me4/Em5-4	0.0414	2.13	Me1/Em2-15 [†]	0.0388	2.32
	Me1/Em6-24	0.0422	2.11	Me4/Em3-13	0.0419	2.25
	Me1/Em3-4 [†]	0.0436	2.08	Me1/Em3-23 [†]	0.0425	2.24
	Me1/Em3-23 [†]	0.0473	2.02	Me1/Em5-10	0.0458	2.18
	Me2/Em6-9	0.0479	2.01	Me1/Em3-4 [†]	0.0462	2.17
				Me2/Em6-4	0.0488	2.12
				Me5/Em5-16	0.0488	2.12
DMY2-Y1	Me2/Em2-16 [†]	0.002	4.86	Me2/Em4-16	0.0007	7.56
	Me4/Em3-11 [†]	0.002	4.84	Me5/Em2-13 [†]	0.0022	6.40
	Me4/Em4-21 [†]	0.0024	4.70	Me1/Em1-17	0.0032	6.00
	Me2/Em3-4 [†]	0.0025	4.66	Me4/Em1-16	0.0047	5.57
	Me1/Em2-20	0.0035	4.40	Me5/Em3-23	0.0067	5.18
	Me5/Em5-6	0.0044	4.21	Me4/Em4-21 [†]	0.0086	4.90
	Me4/Em6-4 [†]	0.0058	3.99	Me4/Em6-7	0.0119	4.54
	Me2/Em5-7	0.007	3.82	Me2/Em1-1	0.0146	4.30
	Me5/Em2-13 [†]	0.0108	3.47	Me4/Em3-11 [†]	0.0153	4.25
	Me1/Em2-15	0.0115	3.41	Me2/Em2-16 [†]	0.0217	3.85
	Me5/Em4-4	0.0149	3.19	Me1/Em3-23	0.0257	3.65
	Me1/Em2-26	0.0158	3.14	Me5/Em1-15	0.0273	3.58
	Me5/Em1-2 [†]	0.021	2.90	Me4/Em4-8	0.0294	3.49
	Me1/Em3-18 [†]	0.0255	2.73	Me5/Em1-2 [†]	0.0332	3.35
	Me4/Em2-12	0.0257	2.72	Me2/Em3-21	0.0351	3.29
	Me5/Em4-7 [†]	0.027	2.68	Me4/Em6-10	0.0362	3.25
	Me1/Em5-11	0.0271	2.67	Me2/Em3-1	0.0365	3.24
	Me4/Em3-7	0.0348	2.46	Me5/Em4-7 [†]	0.0398	3.14
	Me2/Em2-1	0.0426	2.28	Me2/Em3-4 [†]	0.0415	3.09
	Me4/Em4-12	0.043	2.27	Me5/Em3-2	0.0423	3.07
	Me4/Em6-12	0.0469	2.20	Me4/Em3-13	0.0437	3.03
				Me4/Em6-4 [†]	0.0439	3.02
				Me5/Em1-14	0.044	3.02
				Me1/Em3-18 [†]	0.0492	2.89

Table 2- Continued

Trait	Normal environment			Water stress environment		
	Marker	P value	R ² (%)	Marker	P value	R ² (%)
DMY1-Y2	Me5/Em4-4 †	0.0034	3.32	Me1/Em1-17 †	0.00034	4.81
	Me4/Em2-12	0.0041	3.20	Me2/Em4-16	0.0054	3.15
	Me1/Em3-15	0.0086	2.74	Me2/Em5-18	0.01	2.75
	Me5/Em3-1	0.0089	2.72	Me2/Em3-1	0.015	2.48
	Me2/Em2-16	0.0121	2.53	Me5/Em5-16	0.0171	2.39
	Me1/Em1-17 †	0.0132	2.47	Me5/Em4-10	0.0176	2.37
	Me4/Em6-10 †	0.0151	2.38	Me4/Em6-10 †	0.0177	2.37
	Me5/Em1-18	0.0162	2.34	Me5/Em1-15	0.0183	2.35
	Me5/Em5-19	0.0169	2.31	Me1/Em5-23	0.0185	2.34
	Me4/Em1-17	0.019	2.24	Me2/Em4-8	0.0187	2.33
	Me2/Em1-19	0.0206	2.18	Me5/Em1-14	0.0188	2.33
	Me2/Em6-1	0.0234	2.10	Me2/Em1-1	0.0218	2.23
	Me2/Em4-12	0.025	2.06	Me5/Em5-18	0.0268	2.09
	Me4/Em5-19	0.0279	1.99	Me5/Em6-4 †	0.0296	2.02
	Me2/Em5-12	0.029	1.96	Me1/Em1-8	0.0311	1.99
	Me4/Em6-20 †	0.0293	1.95	Me5/Em4-7	0.0345	1.92
	Me1/Em5-11	0.0294	1.95	Me5/Em3-23	0.0389	1.84
	Me4/Em2-26	0.0295	1.95	Me4/Em5-1	0.0397	1.82
	Me1/Em2-26	0.0306	1.93	Me2/Em6-4	0.04	1.82
	Me1/Em2-24	0.0347	1.84	Me4/Em3-16	0.0415	1.79
	Me5/Em6-13	0.044	1.69	Me4/Em6-20 †	0.0439	1.76
	Me5/Em5-2	0.045	1.67	Me5/Em4-4 †	0.0473	1.70
	Me5/Em6-4 †	0.0471	1.64	Me4/Em3-11	0.0481	1.69
	Me1/Em6-16	0.0481	1.63	Me1/Em2-2	0.0488	1.68
				Me4/Em4-8	0.0496	1.67
				Me5/Em5-6	0.0497	1.67
DMY2-Y2	Me1/Em5-11 †	0.0014	6.95	Me5/Em2-20 †	0.0032	5.03
	Me1/Em3-15	0.0028	6.18	Me5/Em3-17	0.0058	4.47
	Me4/Em2-12	0.0058	5.39	Me1/Em4-8 †	0.0061	4.42
	Me1/Em2-1	0.0074	5.12	Me5/Em4-10	0.0064	4.38
	Me5/Em3-4 †	0.0087	4.93	Me1/Em5-11 †	0.0075	4.22
	Me4/Em3-11 †	0.0089	4.90	Me2/Em5-7 †	0.0118	3.80
	Me4/Em6-20	0.0093	4.86	Me2/Em3-21 †	0.0125	3.75
	Me1/Em2-24 †	0.0103	4.74	Me4/Em6-10 †	0.0141	3.63
	Me1/Em2-15 †	0.0114	4.62	Me5/Em3-14 †	0.0176	3.41
	Me5/Em2-20 †	0.012	4.57	Me4/Em2-4	0.0201	3.29
	Me2/Em6-18	0.0142	4.37	Me4/Em6-1	0.0219	3.21
	Me2/Em3-21 †	0.0153	4.28	Me5/Em3-4 †	0.0239	3.12
	Me2/Em2-16	0.0161	4.22	Me1/Em2-24 †	0.0266	3.02
	Me5/Em1-12	0.0179	4.10	Me4/Em1-5	0.0322	2.83
	Me4/Em6-10 †	0.0198	3.98	Me4/Em6-21	0.0334	2.79
	Me2/Em6-1	0.0231	3.80	Me1/Em4-3	0.0338	2.78
	Me1/Em3-20	0.0259	3.67	Me4/Em3-11 †	0.0339	2.78
	Me2/Em5-7 †	0.026	3.66	Me5/Em3-5	0.0349	2.75
	Me3/Em1-11	0.0287	3.55	Me1/Em2-15 †	0.0402	2.61
	Me4/Em3-9	0.0323	3.41	Me5/Em3-23	0.0417	2.58
	Me2/Em3-9	0.0346	3.33	Me1/Em6-9	0.0461	2.48
	Me2/Em3-20	0.0364	3.27	Me2/Em4-7	0.0461	2.48
	Me5/Em4-4	0.0368	3.26	Me2/Em6-7	0.047	2.46
	Me1/Em5-24	0.0373	3.24			
	Me1/Em4-8 †	0.0376	3.23			
	Me5/Em1-18	0.0389	3.19			
	Me1/Em2-11	0.0436	3.06			
	Me2/Em1-3	0.0441	3.04			
	Me5/Em3-14 †	0.0493	2.91			

Table 2- Continued

Trait	Normal environment			Water stress environment		
	Marker	P value	R ² (%)	Marker	P value	R ² (%)
DMY1-Y3	Me2/Em5-12	0.0006	5.88	Me2/Em4-12 [†]	0.0036	3.93
	Me5/Em5-19 [†]	0.0036	4.45	Me5/Em2-20	0.0037	3.92
	Me1/Em1-17 [†]	0.0039	4.39	Me1/Em5-11	0.0068	3.46
	Me5/Em4-11 [†]	0.0056	4.09	Me1/Em1-17 [†]	0.0085	3.30
	Me2/Em4-12 [†]	0.0069	3.91	Me1/Em3-15 [†]	0.0106	3.13
	Me2/Em6-18 [†]	0.0083	3.76	Me5/Em3-22	0.0112	3.09
	Me1/Em2-6	0.0084	3.75	Me2/Em4-7	0.0122	3.03
	Me1/Em3-2 [†]	0.0091	3.68	Me1/Em4-16	0.0133	2.96
	Me5/Em2-15 [†]	0.0093	3.66	Me4/Em6-20 [†]	0.0149	2.87
	Me5/Em4-4 [†]	0.0103	3.58	Me5/Em5-1	0.0189	2.68
	Me5/Em3-8	0.0131	3.37	Me5/Em2-15 [†]	0.0196	2.66
	Me4/Em1-3	0.0175	3.11	Me1/Em2-2	0.0205	2.62
	Me1/Em3-23	0.0179	3.09	Me1/Em3-2 [†]	0.022	2.57
	Me2/Em6-16	0.0192	3.03	Me4/Em6-10	0.0221	2.56
	Me2/Em3-13	0.0214	2.94	Me5/Em5-19 [†]	0.0222	2.56
	Me5/Em6-3	0.0243	2.82	Me4/Em2-23 [†]	0.0251	2.47
	Me5/Em1-18	0.0251	2.79	Me5/Em4-4 [†]	0.0263	2.43
	Me5/Em6-15 [†]	0.0251	2.79	Me5/Em5-18	0.0263	2.43
	Me4/Em2-23 [†]	0.0275	2.71	Me2/Em3-20	0.0294	2.34
	Me4/Em5-16	0.0279	2.70	Me4/Em3-12	0.0303	2.32
	Me2/Em6-5	0.0302	2.63	Me5/Em2-16	0.0326	2.26
	Me4/Em2-12	0.032	2.58	Me5/Em5-16	0.035	2.20
	Me2/Em1-3 [†]	0.0326	2.56	Me5/Em6-15 [†]	0.0359	2.18
	Me2/Em1-2	0.0354	2.49	Me2/Em1-3 [†]	0.0366	2.17
	Me1/Em3-15 [†]	0.0367	2.46	Me4/Em6-12	0.0369	2.16
	Me4/Em5-19	0.0398	2.38	Me5/Em1-17	0.038	2.14
	Me2/Em6-4 [†]	0.0399	2.38	Me2/Em6-4 [†]	0.0387	2.13
	Me5/Em3-4	0.0412	2.35	Me2/Em5-10	0.0407	2.09
	Me4/Em6-20 [†]	0.0433	2.31	Me1/Em1-8	0.0431	2.04
	Me1/Em3-13	0.0435	2.30	Me5/Em4-11 [†]	0.0447	2.01
				Me2/Em1-1	0.0453	2.00
				Me2/Em6-18 [†]	0.0485	1.95
DMY2-Y3	Me1/Em2-1	0.0016	8.88	Me5/Em4-4 [†]	0.000558	9.36
	Me5/Em4-4 [†]	0.0065	6.86	Me1/Em5-11 [†]	0.000997	8.66
	Me4/Em2-12	0.007	6.76	Me4/Em6-10 [†]	0.0021	7.74
	Me2/Em3-4	0.0094	6.33	Me1/Em3-19	0.0033	7.13
	Me4/Em6-10 [†]	0.0114	6.04	Me1/Em3-15 [†]	0.0078	6.01
	Me1/Em5-24	0.0116	6.02	Me4/Em1-13	0.0081	5.95
	Me4/Em2-4	0.0145	5.67	Me5/Em5-18 [†]	0.0082	5.94
	Me4/Em1-3	0.0154	5.59	Me5/Em6-6	0.0105	5.61
	Me5/Em2-20	0.0237	4.93	Me2/Em6-1	0.0118	5.45
	Me2/Em1-19	0.0238	4.92	Me2/Em3-1	0.0153	5.09
	Me4/Em6-20 [†]	0.0253	4.83	Me1/Em2-24 [†]	0.0156	5.06
	Me1/Em4-16 [†]	0.0261	4.78	Me4/Em1-10	0.0178	4.88
	Me2/Em6-7 [†]	0.0268	4.74	Me4/Em6-20 [†]	0.0178	4.88
	Me5/Em5-18 [†]	0.0282	4.66	Me1/Em2-15	0.0192	4.78
	Me2/Em3-21	0.0302	4.56	Me2/Em5-10	0.0218	4.60
	Me1/Em3-15 [†]	0.0307	4.53	Me1/Em5-18	0.0227	4.55
	Me1/Em4-8	0.0308	4.53	Me2/Em1-13	0.0228	4.54
	Me4/Em3-9	0.0337	4.39	Me4/Em2-6 [†]	0.0279	4.26
	Me5/Em3-6	0.0355	4.31	Me5/Em5-6	0.033	4.02
	Me4/Em5-16	0.0382	4.19	Me2/Em6-7 [†]	0.0383	3.81
	Me4/Em4-2	0.0383	4.19	Me2/Em1-12	0.0386	3.80
	Me5/Em5-2	0.0383	4.19	Me1/Em4-16 [†]	0.0474	3.51
	Me1/Em5-11 [†]	0.0427	4.02	Me2/Em4-17	0.0486	3.48

Table 2- Continued

Trait	Normal environment			Water stress environment		
	Marker	P value	R ² (%)	Marker	P value	R ² (%)
RY	Me4/Em3-12	0.0428	4.02			
	Me2/Em4-12	0.043	4.01			
	Me4/Em2-6 [†]	0.0433	4.00			
	Me5/Em1-12	0.0442	3.97			
	Me1/Em1-17	0.0472	3.87			
	Me1/Em2-24 [†]	0.0477	3.85			
	Me4/Em2-12	0.0015	11.17	Me2/Em4-12	0.0041	3.13
	Me2/Em5-2	0.0024	10.41	Me1/Em1-1	0.0068	2.83
	Me1/Em3-19	0.005	9.07	Me4/Em6-12	0.0071	2.80
	Me1/Em2-25	0.0118	7.49	Me1/Em3-15	0.0072	2.79
	Me4/Em6-5	0.0141	7.14	Me1/Em4-15	0.0074	2.78
	Me1/Em4-9	0.0161	6.89	Me1/Em4-7	0.0086	2.68
	Me5/Em1-16	0.0219	6.31	Me5/Em3-5	0.0092	2.64
	Me5/Em6-4	0.0232	6.20	Me4/Em6-20 [†]	0.0104	2.56
	Me2/Em4-14	0.0248	6.07	Me1/Em6-11	0.0208	2.13
	Me2/Em2-3	0.0258	6.00	Me4/Em2-28	0.0226	2.08
	Me2/Em1-19	0.0264	5.95	Me5/Em2-11	0.0238	2.04
	Me1/Em6-1	0.0299	5.71	Me3/Em1-2	0.0286	1.92
	Me4/Em4-10	0.03	5.71	Me1/Em2-4	0.0287	1.92
	Me2/Em1-9	0.0306	5.67	Me1/Em1-8	0.0311	1.87
	Me4/Em6-20 [†]	0.0308	5.66	Me2/Em5-3	0.0355	1.79
DRAD	Me2/Em5-6	0.0327	5.54	Me4/Em2-23	0.0415	1.69
	Me5/Em5-11	0.0338	5.48	Me2/Em6-7	0.0427	1.67
	Me2/Em2-17	0.0358	5.37	Me4/Em1-16	0.0444	1.64
	Me4/Em4-11	0.0361	5.35	Me4/Em6-10	0.0448	1.64
	Me1/Em6-3	0.0395	5.18	Me2/Em1-5	0.0449	1.64
	Me1/Em4-12	0.0427	5.03	Me5/Em2-15	0.045	1.64
	Me4/Em1-3	0.0457	4.90	Me1/Em3-12	0.0482	1.59
	Me5/Em6-12	0.0464	4.87	Me1/Em6-25	0.0486	1.59
				Me5/Em3-22	0.0488	1.58
	Me1/Em4-9	0.000294	9.82	Me4/Em6-12	0.0025	2.25
	Me1/Em3-19	0.000633	8.95	Me2/Em4-12	0.0085	1.77
	Me4/Em4-15	0.0081	5.79	Me1/Em1-1	0.0122	1.62
	Me5/Em6-3	0.0122	5.25	Me5/Em2-20	0.0123	1.61
	Me2/Em5-2	0.0125	5.22	Me5/Em2-11	0.0132	1.58
	Me1/Em6-3	0.0144	5.03	Me4/Em6-10	0.0173	1.47
	Me5/Em1-1	0.0149	4.98	Me5/Em5-9 [†]	0.0197	1.42
	Me4/Em4-10	0.021	4.52	Me2/Em3-4	0.0199	1.42
	Me2/Em3-8	0.0211	4.52	Me1/Em4-8	0.0202	1.41
	Me1/Em6-1	0.0225	4.43	Me2/Em4-6	0.0204	1.41
	Me1/Em1-7	0.0243	4.33	Me1/Em1-17	0.0235	1.35
	Me4/Em2-12	0.0244	4.32	Me1/Em5-16	0.0236	1.34
	Me2/Em1-11	0.0248	4.30	Me5/Em3-5	0.0239	1.34
	Me3/Em1-1	0.0258	4.24	Me1/Em5-11	0.0256	1.31
	Me5/Em1-16	0.0277	4.15	Me3/Em1-2	0.0325	1.21
	Me4/Em6-18	0.0289	4.09	Me4/Em1-17	0.0337	1.20
	Me2/Em4-22	0.0291	4.08	Me1/Em4-7	0.0381	1.14
	Me4/Em4-23	0.031	3.99	Me4/Em2-14	0.0425	1.10
	Me2/Em5-4	0.0317	3.97	Me5/Em4-11	0.0432	1.09
	Me5/Em5-9 [†]	0.0321	3.95	Me4/Em6-20	0.0439	1.08
	Me2/Em1-19	0.0323	3.94	Me1/Em4-15	0.0472	1.05
	Me5/Em6-16	0.0327	3.92	Me4/Em1-13	0.0475	1.05
	Me5/Em3-7	0.0362	3.78	Me1/Em6-11	0.0478	1.05
	Me5/Em1-14	0.0391	3.68	Me1/Em4-16	0.0498	1.03

Table 2- Continued

Trait	Normal environment			Water stress environment		
	Marker	P value	R ² (%)	Marker	P value	R ² (%)
PER	Me4/Em4-6	0.0402	3.64			
	Me4/Em6-19	0.0414	3.60			
	Me5/Em4-4	0.042	3.58			
	Me3/Em1-5	0.0427	3.56			
	Me1/Em6-10	0.0453	3.48			
	Me4/Em6-9	0.0475	3.41			
	Me5/Em5-19 [†]	0.0015	8.63	Me4/Em1-16	0.000013	16.03
	Me5/Em2-3	0.0033	7.57	Me2/Em2-17 [†]	0.000975	10.40
	Me4/Em2-19	0.0044	7.18	Me2/Em1-2 [†]	0.0019	9.42
	Me2/Em3-13	0.005	7.01	Me1/Em1-7	0.0025	9.00
	Me2/Em1-13	0.0056	6.83	Me5/Em5-19 [†]	0.0058	7.67
	Me5/Em6-16 [†]	0.0075	6.43	Me5/Em4-10	0.0062	7.57
	Me5/Em2-15 [†]	0.0111	5.87	Me1/Em3-18 [†]	0.0066	7.46
	Me4/Em3-11 [†]	0.0136	5.57	Me5/Em2-13	0.0084	7.08
	Me4/Em2-28 [†]	0.014	5.53	Me5/Em2-15 [†]	0.015	6.13
	Me1/Em1-22	0.0142	5.51	Me5/Em6-1 [†]	0.0162	6.00
	Me1/Em6-8	0.0159	5.35	Me5/Em6-16 [†]	0.0164	5.98
	Me5/Em6-1 [†]	0.0186	5.11	Me4/Em6-7 [†]	0.0175	5.87
	Me5/Em3-19	0.0238	4.75	Me1/Em4-10	0.0196	5.69
	Me1/Em2-3	0.0239	4.74	Me4/Em3-11 [†]	0.0201	5.64
	Me2/Em1-2 [†]	0.0273	4.55	Me1/Em4-19 [†]	0.0204	5.62
	Me4/Em3-14 [†]	0.0276	4.53	Me4/Em4-8	0.0208	5.59
	Me5/Em4-7 [†]	0.0297	4.42	Me1/Em6-3	0.0244	5.32
	Me2/Em2-14 [†]	0.0303	4.39	Me5/Em4-7 [†]	0.0253	5.26
	Me5/Em5-18	0.0304	4.39	Me4/Em2-5	0.026	5.21
	Me4/Em2-6	0.0315	4.34	Me2/Em2-14 [†]	0.0286	5.06
	Me4/Em1-2	0.0324	4.29	Me4/Em2-28 [†]	0.03	4.98
	Me2/Em2-7	0.0325	4.29	Me2/Em1-18	0.031	4.92
	Me2/Em4-21	0.0332	4.25	Me2/Em1-1	0.0385	4.56
	Me2/Em2-17 [†]	0.0335	4.24	Me2/Em4-20	0.0412	4.45
	Me4/Em6-7 [†]	0.0357	4.15	Me5/Em5-17	0.0414	4.43
	Me5/Em3-22	0.0375	4.07	Me4/Em3-14 [†]	0.0469	4.23
	Me1/Em4-19 [†]	0.038	4.06	Me1/Em3-4	0.0474	4.21
	Me1/Em6-17	0.0384	4.04			
	Me4/Em5-16	0.0419	3.91			
	Me2/Em4-17	0.0432	3.86			
	Me1/Em3-18 [†]	0.0454	3.79			
SDI-Y1	Me5/Em4-12	0.0000935	11.38			
	Me4/Em5-5	0.000993	8.66			
	Me2/Em3-22	0.0012	8.41			
	Me2/Em1-13	0.004	6.89			
	Me4/Em4-19	0.0051	6.59			
	Me1/Em5-24	0.0071	6.13			
	Me1/Em2-17	0.0075	6.06			
	Me1/Em3-21	0.0075	6.06			
	Me2/Em6-9	0.0107	5.58			
	Me4/Em2-20	0.0114	5.50			
	Me5/Em6-10	0.0138	5.24			
	Me4/Em3-6	0.0155	5.07			
	Me2/Em6-14	0.0171	4.94			
	Me5/Em6-7	0.0173	4.92			
	Me4/Em4-8	0.0193	4.77			
	Me4/Em2-5	0.0197	4.75			
	Me1/Em6-14	0.0215	4.63			

Table 2- Continued

Trait	Normal environment			Water stress environment		
	Marker	P value	R ² (%)	Marker	P value	R ² (%)
	Me4/Em4-18	0.0218	4.60			
	Me4/Em5-7	0.027	4.31			
	Me2/Em5-10	0.0294	4.18			
	Me5/Em1-6	0.0321	4.06			
	Me2/Em2-4	0.0374	3.85			
	Me2/Em4-18	0.0384	3.81			
	Me2/Em4-24	0.0394	3.77			
	Me2/Em1-19	0.0418	3.69			
	Me2/Em1-7	0.042	3.69			
	Me4/Em5-19	0.0438	3.63			
	Me1/Em2-11	0.0449	3.59			
	Me3/Em1-12	0.0469	3.53			
	Me5/Em4-18	0.0469	3.53			
SDI-Y2	Me5/Em1-8	0.0049	3.87			
	Me2/Em4-20	0.0052	3.83			
	Me5/Em3-18	0.0097	3.33			
	Me1/Em2-23	0.0103	3.29			
	Me5/Em1-25	0.0109	3.24			
	Me2/Em6-12	0.0117	3.19			
	Me1/Em6-16	0.0123	3.14			
	Me1/Em6-7	0.0128	3.11			
	Me4/Em3-3	0.0137	3.06			
	Me2/Em4-5	0.0162	2.93			
	Me2/Em6-4	0.0163	2.92			
	Me4/Em3-5	0.0178	2.85			
	Me2/Em6-3	0.0182	2.83			
	Me2/Em3-12	0.0211	2.71			
	Me5/Em1-6	0.022	2.68			
	Me2/Em1-6	0.0238	2.61			
	Me1/Em5-23	0.029	2.45			
	Me4/Em4-8	0.0299	2.43			
	Me4/Em5-14	0.0305	2.41			
	Me1/Em5-1	0.0326	2.36			
	Me4/Em4-3	0.0331	2.34			
	Me5/Em2-12	0.0332	2.34			
	Me1/Em4-13	0.036	2.27			
	Me5/Em3-5	0.0436	2.12			
	Me1/Em2-13	0.045	2.09			
	Me4/Em5-19	0.0452	2.09			
	Me4/Em6-3	0.0471	2.06			
	Me4/Em1-17	0.0489	2.02			
	Me2/Em1-3	0.0494	2.02			
SDI-Y3	Me5/Em3-6	0.00006	8.61			
	Me1/Em2-20	0.00009	8.34			
	Me4/Em6-4	0.00079	6.53			
	Me4/Em2-4	0.0018	5.80			
	Me4/Em5-5	0.0021	5.63			
	Me1/Em1-20	0.0022	5.62			
	Me1/Em6-21	0.0066	4.56			
	Me2/Em5-8	0.0088	4.27			
	Me4/Em2-25	0.0092	4.23			
	Me5/Em2-3	0.0095	4.20			
	Me2/Em5-7	0.011	4.05			
	Me1/Em3-2	0.0121	3.96			

Table 2- Continued

Trait	Normal environment			Water stress environment		
	Marker	P value	R ² (%)	Marker	P value	R ² (%)
	Me2/Em4-16	0.0128	3.90			
	Me4/Em2-11	0.0131	3.88			
	Me5/Em5-14	0.0145	3.77			
	Me1/Em4-8	0.0156	3.70			
	Me5/Em3-13	0.0167	3.64			
	Me5/Em5-3	0.0182	3.55			
	Me2/Em6-5	0.0193	3.49			
	Me4/Em2-15	0.0199	3.45			
	Me4/Em1-18	0.0234	3.29			
	Me5/Em5-17	0.0242	3.26			
	Me5/Em2-10	0.0249	3.23			
	Me4/Em2-3	0.0254	3.21			
	Me4/Em6-16	0.0254	3.21			
	Me4/Em2-10	0.0276	3.12			
	Me4/Em2-6	0.0287	3.08			
	Me1/Em3-8	0.0291	3.07			
	Me1/Em1-12	0.0302	3.03			
	Me1/Em4-11	0.0318	2.98			
	Me4/Em5-11	0.0331	2.94			
	Me2/Em2-4	0.0362	2.85			
	Me5/Em3-20	0.0417	2.70			
	Me5/Em4-5	0.0426	2.68			
	Me1/Em1-18	0.0438	2.65			

† Stable markers under normal and water stress environment

Figure legends

Fig. 1- Population structure analysis in a diversity panel of smooth bromegrass genotypes (Δk was used to determine the optimum k value for population structure using the Bayesian clustering method).

Fig. 2- Genetic relatedness of smooth bromegrass genotypes analyzed by STRUCTURE program. Numbers on the y-axis indicate the membership coefficient. The color of the bar indicates the five sub-groups identified through the STRUCTURE program. Genotypes with the same color belong to the same group.

$$\text{DeltaK} = \text{mean}(|L''(K)|) / \text{sd}(L(K))$$