



Genotypic response on stability for yield and nitrogen use efficiency in triticale (*X Triticosecale* Wittmack) under different nitrogen regimes

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Abstract

Nitrogen (N) has major roles in plant physiology thus in crop yield and quality. Use of N efficiently in diverse environments has an importance for both economic and environmental friendly agricultural production. This study was carried out to evaluate the biomass and grain yield capacity of triticale (*X Triticosecale* Wittmack) genotypes and performance of nitrogen use efficiency (NUE) traits at different N rates and year, investigate the relative importance of genotypic variance components and heritability of yield and NUE traits, estimate the "genotype × environment" interactions and the stability of these interactions. Eleven triticale genotypes (six cultivars Karma, Melez, Mikham, Presto, Samursortu, Tatlicak, and five lines TVD-3, TVD-4, KTVD-9, TVD-17, TVD-25) were grown with three N rates (40, 80 and 160 kg N ha⁻¹) in two subsequent growing seasons. The experimental design was a split-plot design with four replicates. The calculated NUE indexes were agronomic efficiency, physiological efficiency, agro-physiological efficiency, apparent recovery efficiency and utilization efficiency for the triticale genotypes used in the experiment. It has been determined that eleven triticale genotypes differed for stability for biomass and yield, protein content and N efficiency traits examined under different environmental conditions. TVD-3, TVD-4 and TVD-17 genotypes were considered the best in terms of adaptation to all environments for NUE traits. Samursortu and TVD-25 were found to be the most stable for biomass and grain yield while any genotype was not found to be stable for protein content.

Key words: grain, genotype x environment, protein, biomass, N utilization

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Farklı azot rejimlerinde tritikalenin (*X Triticosecale* Wittmack) verim ve azot kullanım etkinliğinin stabilitesine genotipik yanıt

Özet

Azot (N), bitki fizyolojisi, ürün verimi ve kalitesinde önemli rol oynar. Azotun çeşitli çevrelerde etkin bir şekilde kullanılması, hem ekonomik hem de çevre dostu tarımsal üretim için önemlidir. Bu çalışma, tritikale (*X Triticosecale* Wittmack) genotiplerinin farklı N oranları ve yıllarda, biyokütle ve tane verimi kapasitesini aynı zamanda N kullanım etkinliği (NUE) özelliklerini değerlendirmek, verim ve NUE özelliklerinin genotipik varyans bileşenleri ve kalıtsallığının önemini araştırmak, bu özellikler için "genotip × çevre" etkileşimleri ve bu etkileşimlerin stabilitesini belirlemek amacıyla yürütülmüştür. Onbir triticale genotipi (altı çeşit; Karma, Melez, Mikham, Presto, Samursortu, Tatlıcak ve beş hat; TVD-3, TVD-4, KTVD-9, TVD-17, TVD-25), üç N dozu (40, 80 ve 160 kg N ha⁻¹) ve iki ardışık deneme sezonunda yetiştirilmiştir. Deneysel tasarım, dört tekrarlamalı bölünmüş parseller deneme desenine göre oluşturulmuştur. Tritikale genotiplerinin NUE indeksleri (agronomik etkinlik, fizyolojik etkinlik, agro-fizyolojik etkinlik N değerlendirme etkinliği ve N kullanım) hesaplanmıştır. Onbir triticale genotipinin farklı çevresel koşullar altında incelenen biyokütle, tane verimi, protein içeriği ve NUE özellikleri açısından istikrarı bakımından farklı olduğu belirlenmiştir. NUE özellikleri için tüm çevrelerde adaptasyon açısından TVD-3, TVD-4 ve TVD-17 genotipleri en iyi olarak kabul edilmiştir. Biyokütle ve tane verimi açısından Samur sortu ve TVD-25 genotiplerinin stabil oldukları belirlenirken, protein içeriği için hiçbir genotip stabil bulunmamıştır.

Anahtar kelimeler: tane, genotip x çevre, protein, biyokütle, azottan yararlanma

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1. Introduction

Environment consists of the factors such as climate conditions, region and agronomical applications including fertilizer applications and management which affect plant development. The environmental conditions can influence negative or positive crop genotypes growth and yield performance. Genotypes \times environment interactions are highly significant in the growing and breeding of plant varieties because they can decrease genotypic stability values in different environments (Hebert et al., 1995). The stability was described as an adaptation of genotypes to unpredictable and temporary environmental conditions and a method has been developed to select stable genotypes which are unaffected or less affected by environmental changes (Allard and Bradshaw, 1964).

Yield stability always has been most attractive trait of all plant breeding programs because of the fluctuations in the mean annual yield, especially in the arid and semi-arid areas (Mohammadi et al., 2012). A new developed cultivar is released for production to farmers after testing genotypes in multi-environments because plant producers are most interested in a cultivar that gives consistent yields under different growing conditions (Naghavi et al., 2010; Sayar et al., 2013). Besides developing yield stability with different environmental conditions, improving nitrogen (N) use-efficient genotypes should be a significant aim for plant breeders. The reason behind this is applying the high rates of N fertilizers can cause the environmental pollution and economic loss (Al-Naggar et al., 2015) such as leaching of N and causing eutrophication of water (Vitousek et al., 1997) and increasing emissions of the greenhouse gas nitrous oxide (N₂O) from agricultural soils (Bouwman et al., 2002). If the cultivars which absorb N better are developed, both high crop yield can be gained with low fertilizer rates and pollution risks can be limited.

To determine the maximum grain and biomass production per unit of applied N, indexes of N use efficiency (NUE) are defined (Moll et al., 1982; Fageria and Baligar, 2001). NUE is usually dependent on interactions of genotype and environmental factors such as nutrient balance, water availability and applied fertilizer rate. Developing and identifying superior N-efficient genotypes is necessary to overcome economic and environmental concerns, and lack of adoption of more efficient N management strategies (Singh et al., 1998). Previous researchers remarked that N uptake and use efficiency were influenced by genotypic structure. They stated how crop yield was affected by different N rates (Gaju et al., 2011; Lemes da Silva et al., 2014; Hitz, 2015; Todeschini et al., 2016). Yi et al. (2011) reported that NUE and correlative indexes of 31 triticale genotypes were investigated at the tillering, jointing and heading stages under low and high N supplies. They found that triticale genotypes showed differences for NUE as high, low and middle NUE genotypes and the plant height and shoot biomass were significantly correlated with the NUE and suggested these as the indirect index to evaluate NUE of triticale. However, there is no stability study for triticale in different N rates and climate conditions and whether there is any consistency between yield stability and heritability of NUE traits of different triticale genotypes under diverse environmental conditions.

The purpose of this study were to (1) evaluate the biomass and seed yield capacity of triticale genotypes and performance of NUE traits at different N rates and year; (2) investigate the relative importance of genotypic variance components and heritability of yield and NUE traits; (3) estimate the genotype \times environment interactions and the stability of these interactions using Eberhart and Russell's stability parameters under varying N environments.

2. Materials and methods

The field experiment was conducted at Faculty of Agriculture, Eskisehir Osmangazi University, Turkey, with N rates and triticale cultivars in the years of 2005/2006 and 2006/2007. The average annual precipitation and temperature (from October to July) for the area over the last 60 years and experimental years were presented in Figure 1. Soil samples (0-30 cm) were taken at sowing time and were air-dried, passed through a 2 mm sieve and analyzed for physical and chemical parameters using a standard procedure (Rowell 1996). Soil homogeneity was taken into consideration when the experiment design was established. The soil samples were analysed for texture (hydrometer method), organic matter (Walkley-Black method), pH (1:2.5 soil:water), lime (Scheibler calcimeter), total N (Kjeldahl method) and electrical conductivity (EC). Plant available Fe, Cu, Mn, and Zn concentrations were determined using method described by Lindsay and Norwell (1978) with an atomic absorption spectrometer (Analytik Jena novAA 350, Jena, Germany). Soil properties of research area across two years were similar with low organic matter (1.78%), alkaline (8.0), and moderate lime (6.1%). The available phosphorus and potassium of the soils were 8.1 and 198 mg kg⁻¹, respectively. The soils contained insufficient Zn (0.17 mg kg⁻¹), Mn (10.3 mg kg⁻¹) and sufficient Cu (1.27 mg kg⁻¹) but high Fe (4.55 mg kg⁻¹) concentrations. The soil texture was sandy clay loam and the soils had low in N (0.03%) supply to plants.

The four N rates (control, 40 (N₄₀), 80 (N₈₀) and 160 (N₁₆₀) kg N ha⁻¹) were applied to 11 hexaploid triticale genotypes (six cultivars Karma, Melez, Mikham, Presto, Samur sortu, Tatlicak, and five lines TVD-3, TVD-4, KTVD-9, TVD-17, TVD-25). The seeds were obtained from Bahri Dagdas International Agricultural Research Institute in Konya. The plots were arranged with 6 rows at 25 cm spaces. The 450 seeds per meter square were planted in October. The plants were fertilized with 60 kg P₂O₅ ha⁻¹ as a triple superphosphate to all plots at sowing time. A split-plot with four replicates was designed. The N rates consisted of the main plots and triticale genotypes were sown as subplots. Nitrogen was not applied to control (N₀) plots. The half of the N rates was applied as an ammonium sulfate (26% N) at

planting while the rest of the N was treated as topdressing using ammonium nitrate (33% N) at tillering stage. The harvests were done in July.

Plants aboveground were removed at harvest for measuring of N concentration in grain and straw using by the Kjeldahl digestion method. The total N uptake was calculated by multiplying the dry weight by the N concentration in the grain and straw. The dry matter (biomass) and seed weight were weighted by drying the sampled plants.

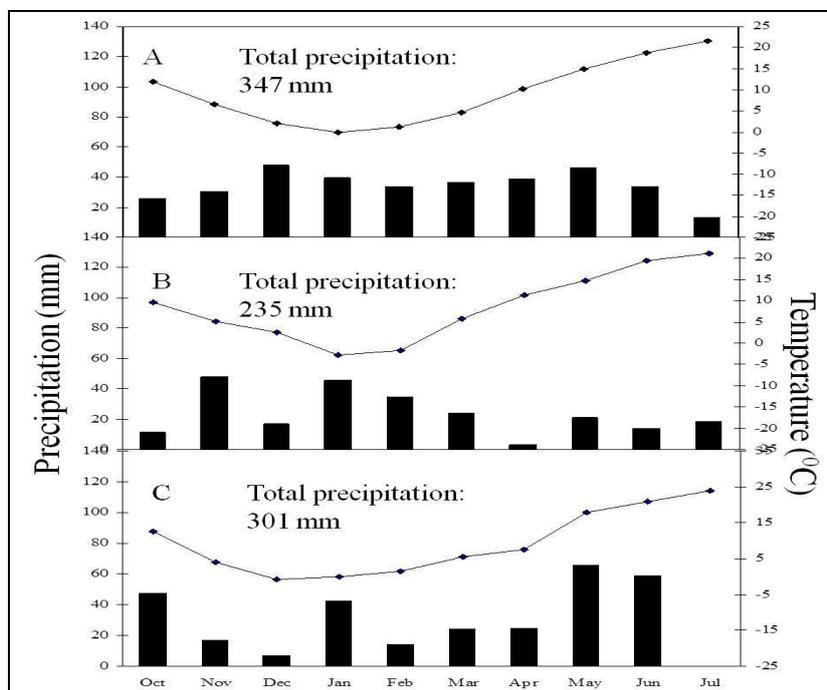


Figure 1. Total precipitation and temperature for 30-year average (A) and for 2005/06 (B), 2006/07 (C) at experiment field.

The NUE indexes (kg kg^{-1}) were calculated according to Fageria and Baligar (2001): (i) agronomic efficiency (the ratio of the increase in grain yield over N-control plots to the applied rate of N), (ii) physiological efficiency (the ratio of the increase in biomass yield over N-control plots to the increase in nutrient accumulation over N-control plots), (iii) agrophysiological efficiency (the ratio of the increase in grain yield over N-control plots to the increase in nutrient accumulation over N-control plots), (iv) apparent recovery efficiency (the ratio of the increase in nutrient accumulation over N-control plots to the applied rate of N \times 100) (v) utilization efficiency (the multiplying of agro-physiological efficiency and apparent recovery efficiency).

The six environments (named E₁; 40 N kg ha⁻¹, E₂; 80 N kg ha⁻¹; E₃; 160 N kg ha⁻¹ for 2005/2006 and the other three environments; E₄; 40 N kg ha⁻¹, E₅; 80 N kg ha⁻¹; E₆; 160 N kg ha⁻¹ for 2006/2007) for statistical analysis were occurred from calculated NUE of triticale genotypes. A combined variance analysis was performed across the test environments. Broad sense heritability (H%) and variance components for examined traits were calculated as suggested by Demir and Turgut (1999). The linear regression model was used to describe genotypic stability (Eberhart and Russell 1966). All statistical analyses were performed using the SAS (Statistical Analyses Systems) program (SAS Institute 1999).

3. Results

A combined variance analysis of examined traits of the 11 triticale genotypes tested across six environments (Table 1) showed that 'environment', 'genotypes' and 'genotype \times environment' interactions were highly significant ($p < 0.01$). While significant variations of 'genotype' and 'environment' indicated the existence of variability among the tested cultivars for all traits, highly significant 'genotype \times environment' interactions suggested differential response of cultivars across testing environments and the need for stability analysis. This is an evidence that there were significant genetic background variations among triticale genotypes and the response of examined traits. Also, important variations were shown due to combination of N fertilization and climate changes over the years (environments). Many researchers (Anbessa et al., 2009; Sadras and Slafer, 2012; Lemes da Silva et al., 2014; Abd El Mohsen and Amein, 2016; Katar et al., 2016) agree with the finding that yield and agronomic traits are affected by genotypes, environmental factors and their interactions. Genotypic variance was smaller than environmental variance for all traits except grain yield (Table 1). This result indicated that these traits are influenced by environmental changes. High value of 'genotype \times environment' interaction variance proved this opinion. The dominance of the environmental changes caused increased

phenotypic variance and decreased broad sense heritability degree for all traits (Table 1). The broad sense heritability (H%) was 69.0 for grain yield, although grain yield is a complex trait and many of them are affected by changes of environmental factors. For agronomic efficiency, physiological efficiency, agro-physiological efficiency, apparent recovery efficiency and utilization efficiency traits, small broad sense heritability pointed out that N fertilizer rates and climate changes must be considered besides selection according to genotype. To develop cultivars able to take up N from soils and fertilizers and to use it to produce more grain is a good way for improving NUE in cereals. However, one of the problems here is low degree of heritability. Another way improves N-efficiency in cereals is to have a better fertilizer management. The fertilizer efficiency can be improved by combining applications considering crop demand and climate changes (Barraclough et al., 2010). Climate has a major influence on N demand (crop growth and grain yield), and on the N supply (availability of N in soil and/or fertilizer). Presences of 'genotype x environment' interactions is a major obstacle to making proper fertilizer recommendations. Therefore, it is important to identify genotypes that remain the stable under changing climatic conditions and N applications.

Determining of stable triticale genotypes adapted to changeable environments is possible with the differential ranking of genotypes' performance across environments (Baker, 1988). The mean values of examined traits for 11 triticale genotypes tested across six environments are presented in Table 1. The means of all traits across the six environments showed substantial changes reflecting the presence of high 'genotype x environment' interactions. Mean agronomic efficiency, physiological efficiency, agro-physiological efficiency, apparent recovery efficiency and utilization efficiency varied from 3.04 (E₃)- 17.08 (E₁) kg kg⁻¹, 32.96 (E₆)- 78.34 (E₄) kg kg⁻¹, 7.50 (E₃)- 24.30 (E₁) kg kg⁻¹, 35.57 (E₆)- 75.11 (E₄) % and 12.55 (E₆)- 59.52 (E₄) kg kg⁻¹ respectively. While grain yield and biological yield ranged from 2.90 (E₄)- 3.63 (E₂) and 16.62 (E₆)- 19.97 (E₂) ton ha⁻¹, the lowest and highest grain protein content values were gained from E₄ (2.90%) and E₂ (3.63%), respectively. Otherwise total N accumulation values were close to each other (Table 1).

Regression coefficients of genotypes versus the mean values of examined traits were illustrated (Figure 2). The mean agronomic efficiency of the 11 triticale genotypes ranged from 6.9 (Karma) kg kg⁻¹ to 14.6 (Mikham) kg kg⁻¹. The highest physiological efficiency value was obtained from Samursortu (74.40 kg kg⁻¹). For agro-physiological efficiency, apparent recovery efficiency and utilization efficiency, the highest values were determined as 17.4 kg kg⁻¹ (Presto), 74.4% (Mikham) and 48.6 kg kg⁻¹ (Samursortu), respectively. The lowest utilization efficiency value was obtained from Karma as 26.1 kg kg⁻¹ (Figure 2). Regression coefficients ranged between 0.65-1.21 for agronomic efficiency, 0.20-1.70 for physiological efficiency, 0.30-1.70 for agro-physiological efficiency, 0.60-1.60 for apparent recovery efficiency and 0.50-1.50 for utilization efficiency, respectively (Figure 2).

In addition, the mean values of total N accumulation were close to each other (0.14-0.17 ton ha⁻¹). Mean values of biomass and grain yield changed between 16.68 (Tatlicak)- 20.11 (TVD-25) ton ha⁻¹ and 2.33 (KTVD-9)-3.88 (TVD-25), respectively. In addition, Tatlicak had the lowest grain protein content with 14.55% and TVD-25 had the highest grain protein content with 16.79 (Figure 2). Regression coefficients ranged between 0.49-1.65 for total N accumulation, 0.71-1.74 for biomass yield, -0.42-1.58 for grain yield and 0.15-1.57 for protein content, respectively (Figure 2). This broad variation in regression coefficients reflects the different responses of different genotypes to environmental changes. Akcura et al. (2005) and Sayar et al. (2013) found that regression coefficients (bi values) ranging from 0.46 to 1.56 for grain yield in wheat genotypes and 0.283 to 1.325 for dry-matter yield in vetch genotypes at different environmental conditions and they stated that the genotypes responses could be differed to environmental changes.

Eberhart and Russell (1966) expressed that regression coefficients (bi) approximating 1.0 combined with small value of S²d (nearly zero) point out an average stability. When the regression coefficient approximating 1 is associated with the high mean values of traits, genotypes have general adaptability and when associated with low mean values of traits, genotypes are poorly adapted to all environments (Eberhart and Russell 1966). If regression values are above 1.0, it is considered that genotypes have below average stability with higher sensitivity to environmental change. So, such genotypes should be proposed for high yielding environments. Conversely, when regression coefficients decrease below 1.0, genotypes provide more tolerance to environmental change and increase adaptability to low yielding environments with above average stability (Eberhart and Russell, 1966; Akçura et al., 2006).

If Figure 2 is examined from this perspective, it is seen clearly that the cultivars Mikham and Samursortu had high mean performance and regression coefficient value for N utilization efficiency traits. So, these genotypes were regarded as sensitive to environmental changes and can be recommended for favorable conditions (high N rates). Genotypes KTVD-9 and Karma had less than unity of regression coefficient and had low mean values of N utilization efficiency traits. These genotypes are, therefore, insensitive to environmental changes and have adapted to the poor environments (low N rates). With its high mean values of N utilization efficiency traits and a regression coefficient that did not differ significantly from 1.0, TVD-3, TVD-4 and TVD-17 genotypes showed better adaptability to all environmental conditions. These genotypes can be described as the most stable genotypes for N utilization efficiency traits. This data is fundamental to understand the correlative role of genotypic variation on N utilization efficiency in different environments and the consequences of N fertilizer rates interactions on ecosystem processes. The results from Pregitzer's (2010) study show that across an environmental gradient *Populus angustifolia* genotypes can influence N mineralization through feedbacks between environmental variation, tree phenotype and soils. This result supported our opinion.

Table 1. Mean performance, variance analysis and variance components for examined traits of 11 triticale genotypes tested across 6 environments

	Agronomic efficiency	Physiological efficiency	Agro-physiological efficiency	Apparent recovery efficiency	Utilization efficiency	Total N accumulation	Grain yield	Biological yield	Grain protein content
E ₁	17,08	62,62	24,3	71,91	46,57	0,14	3,46	18,21	13,47
E ₂	10,71	63,25	15,12	72,58	45,25	0,16	3,63	19,97	14,76
E ₃	3,04	48,7	7,5	39,96	19,52	0,17	3,26	19,47	15,94
E ₄	8,65	78,34	11,43	75,11	59,52	0,15	2,9	17	15,5
E ₅	9,24	53,44	12,04	74,44	41,49	0,18	3,3	17,93	16,64
E ₆	3,68	32,96	9,49	35,57	12,55	0,18	3,15	16,62	17,49
Variance Analysis									
Environment(E)	1159,69**	10422,4**	1561,73**	15136,0**	13984,4**	0,015**	2,79**	76,88**	87,88**
Genotype (G)	162,38**	1994,7**	308,14**	821,8**	1133,0**	0,005**	4,03**	33,83**	14,36**
GxE	59,47**	1609,9**	139,77**	745,1**	1052,2**	0,001**	0,35**	4,87**	4,62**
Variance Components									
GV	4.29	16.02	7.01	3.18	3.36	0,0002	0,15	1,21	0,40
EV	25.01	200,28	32.32	327.06	293.91	0,0003	0,05	1,64	1,89
GxEV	14.86	402.40	34.94	186.20	263.25	0,0002	0,09	1,21	1,15
PV	31.77	283.38	45.16	361.29	341.12	0,001	0,22	3,04	2,49
H _{bs}	0.15	0.06	0.16	0.01	0.01	0,32	0,69	0,40	0.16

*p≤0.05, **p≤0.01, (GV: genotypic variance, EV: environmental variance, PV: phenotypic variance, H_{bs}: broad sense heritability)

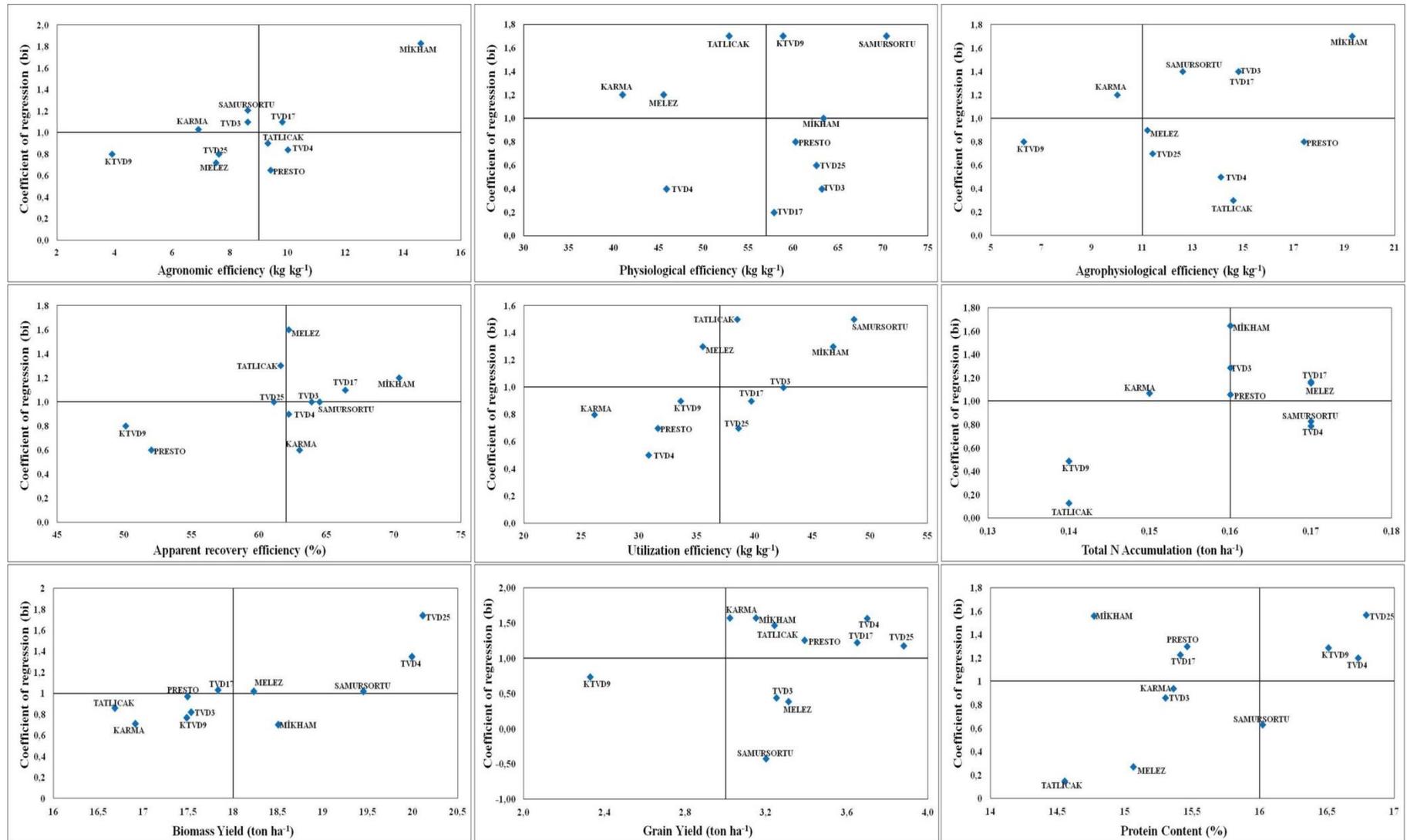


Figure 2. The relationship between the regression coefficients and mean value of examined traits for 11 triticale genotypes. The horizontal solid line represents the mean coefficient of regression and the vertical solid line denotes the mean value of examined traits.

Genotypes TVD-25, TVD-3 and Mikham had regression coefficients highly greater than unity for total N accumulation over and equal mean values (Figure 2). For this reason, these genotypes are sensitive to environmental changes and can be suggested for growing under favorable conditions for high N accumulation. KTVD-9 and Tatlicak were determined as genotypes had poor adaptability to unfavorable environmental conditions with below unity b_i value and low total N accumulation values. The other genotypes showed average stability for total N accumulation. Karma for biomass yield was insensitive to environmental changes and have adapted to the poor environments because it was less than unity ($b_i = 1.0$) regression coefficient and had low mean value. On the other hand, Samursortu could be considered as the most stable genotype for biomass yield (Figure 2). Although TVD-25 had regression coefficients remarkably greater than unity and over mean value for biomass yield, it had a high mean value, a regression coefficient equal to the unity ($b_i = 1$) for grain yield. Therefore, this genotype was sensitive to environmental changes and could be recommended for cultivation under favorable conditions for biomass yield. In contrast, the same genotype could be considered as stable for grain yield. Samursortu, TVD-3, Melez were well adapted to unfavorable environmental conditions due to less than unity ($b_i = 1.0$) and had over mean grain yields. According to Figure 2, it could be concluded that Tatlicak's grain protein content would be affected from environmental changes. TVD-25, KTVD-9 and TVD-4 in favorable conditions give high grain protein content. Moreover, Samursortu's grain protein content is insensitive to environmental changes and have average adaptability to unfavorable environmental conditions

4. Conclusions and discussion

There were highly significant 'genotype x environment' interactions for all traits examined. Especially, the heritability of N use efficiency traits was very small and thus it was concluded that these traits are highly affected from environmental conditions. E1 and E4 (40 N rates) were determined as the best environment for N efficiency traits, however E2 (80 N rates) were the best environment for yield and protein content. TVD-3, TVD-4 and TVD-17 genotypes were considered the best in terms of adaptation to all environments for N utilization efficiency traits. In that case, these genotypes well respond even the low N fertilizer rates. Samursortu and TVD-25 were found to be the most stable for biomass and grain yield. Any genotype was not found to stable for protein content, however, Samursortu may be satisfactory with average adaptability and TVD-25 can be gained high grain protein content under favorable environments. For these genotypes, optimum N fertilizer rates (80 N) would be appropriate for high yield and protein content. Stable genotypes were different for N utilization efficiency traits and yield. This inconsistent values reflect the importance of understanding the type and magnitude of 'genotype x environment' interaction for N utilization efficiency traits in triticale production and breeding programs carried out under different environmental conditions in order to select a highly performance and genotypically stable genotype. Success in breeding studies would be improved by the selection of 'genotype x environment' according to N utilization efficiency traits besides yield stability. Such studies may be useful to develop flexible fertilizer management advice for farmers in diverse environments.

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