

## **Involvement of Chromosome 5R Carrying the Genes Controlling Yield and Yield Stability in Rye (*Secale Cereale* Cv. Imperial)**

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**Abstract.** Identification of the genetic architecture of phenotypic stability and management of adaptational genes is a prerequisite for improvement of adaptation. To locate the genes controlling adaptation, disomic addition lines of Rye into the genetic background of Chinese Spring were used in a randomized complete block design with three replications under two different conditions (rainfed and irrigated) for three years. Combined analysis of variance showed highly significant differences for genotypes (G), environments (E) and GE interaction indicating variability between genotypes, environments and their effects in the GE interaction and possible chromosomal localization of the genes controlling yield and yield stability in Rye. The results of regression analysis showed that linear GE interaction accounted for 13% of the variability in the GE interaction, While AMMI1 and AMMI2 accounted for 86% of GE interaction. Yield stability index (YSI) which incorporate AMMI stability value (ASV) and mean yield in a single non- parametric index revealed that most of the genes controlling yield and yield stability in Rye are located on chromosome 5R

**Keywords:** AMMI Model, Disomic Addition Lines, Gene Location, Yield Stability Index

### **1. Introduction**

The genotype by environment interaction is a major problem in the study of quantitative traits because it complicates the interpretation of genetic experiments and makes predictions difficult. Therefore, the first goal of plant breeders in a crop breeding program is the development of cultivars or genotypes which are stable or adapted to a wide range of diversified environments (Farshadfar and Sutka, 2006; Abdulahi et al., 2009; Pimsaen et al., 2010).

GE interaction reduces the association between genotypic and phenotypic values and thereby reduces the genetic progress resulted from selection (Kearsey and Pooni, 2004). In semi-arid areas where climate is unpredictable, production of varieties with high yield and wide adaptation is one of the most important goals of plant breeding programs. When discussing unpredictable changes in the yield we use the term phenotypic stability which is the changes occurred in the phenotypic expression of yield (Becker and Leon, 1988).

Different methods and techniques have been used for the measurement of genotypic stability which are reviewed else where ( Lin et al., 1986; Westcott, 1986; Becker and Leon, 1988; Crossa et al., 1990). Regardless of the definition of stability parameter one important question is whether stability is heritable or

not? If stability is not heritable, then using this parameter in breeding programs is fruitless (Lin and Binn, 1991, 1994; Jalata et al., 2011). If stability is heritable, the next step in the genetic analysis is identification of the chromosomal location of the genes controlling adaptation (Farshadfar, 2008). To understand the genetics of continuous variation, it is necessary to identify the chromosomal location of the genes controlling quantitative attributes such as yield and yield stability (Eskridge et al., 2000).

Various techniques (biometrical, cytogenetic and molecular) have been used to locate the genes monitoring quantitative traits among which cytogenetic methods (monosomic, disomic, substitution and disomic addition analysis) have been widely used. Because of the complex nature of phenotypic stability, very little information is available on the chromosomal location of the genes conditioning adaptation (Morgan, 1991; Koszegi et al., 1996; Farshadfar and Sutka, 2003).

Species related to wheat, including both distantly related and progenitor species, represent a large reservoir of useful variability that can be exploited in wheat improvement (Jiang et al., 1994; Friebe, 1996). They contain indispensable genes required for wheat improvement especially under an unfavourable environment. They generally have tolerance to biotic and abiotic stresses and survive under low input conditions. Disomic addition lines (DALs), in which a single pair of homologous chromosomes from a related species are added to the full chromosome complement of the recipient, are valuable materials to identify alien chromosomes carrying useful genes and prepare the starting point for gene transfer and genetic improvement of genotypic stability (Gale and Miller, 1987; Ellis et al., 2000). Therefore, present investigation was carried out to identify the chromosome(s) most probably carrying the QTLs controlling yield and yield stability in Rye.

## 2. Materials and Methods

To locate QTLs controlling yield and yield stability, 7 disomic addition lines (1R to 7R) of *Secale cereale* cv. Imperial (2n=2x=14) into the genetic background of Chinese Spring (CS= recipient) wheat (2n=6x=42) and a check (*Triticum aestivum* L. cv. Sardary = SAR; a stable landrace from west of Iran) and Rye variety Imperial (RIM = donor) together with Rye variety Lovaspatonai (RLO) were used in 6 rainfed and irrigated conditions in the College of Agriculture, Razi University, Kermanshah, Iran (47° 20' N latitude, 34° 20' E longitude and 1351.6 m altitude). Climate in the region is classified as semiarid with mean annual rainfall of 378 mm. Minimum and maximum temperature at the research station were -27°C and 44°C, respectively. The experimental design for each environment was a completely randomized block design with three replications. The plots consisted of 2m and at 15×25 cm inter-plant and inter-row distances, respectively. Each plot consisted of 100 seeds (each row 50 seeds). The environments were considered as random factors and genotypes as fixed factors. At the time of harvesting 5 single plants were selected randomly and grain yield was measured.

### 2.1. AMMI Analysis

Additive main effect and multiplicative interaction (AMMI) was performed using IRRISTAT software. Briefly, analysis of variance is used to partition variance into three components: genotype deviations from the grand mean, environmental deviations from the grand mean, and GE deviations from the grand mean. Subsequently, multiplication effect analysis is used to partition GE deviations into different interaction principal component axes (IPCA), which can be test for statistical significant through ANOVA. The AMMI analysis is interpreted by plotting the IPCAs of GE in various types of biplots.

### 2.2. AMMI Stability Value (ASV)

ASV is the distance from the coordinate point to the origin in a two- dimensional scattergram of IPCA1 scores against IPCA2 scores in the AMMI model and is calculated as follows (Purchase et al., 2000).

$$ASV_i = \sqrt{\left[ \frac{SS_{IPCA1}}{SS_{IPCA2}} (IPCA1score) \right]^2 + (IPCA2score)^2}$$

SSIPC1/SSIPC2 is the weight given to the IPC1 value by dividing the IPC1 sum of square on the IPC2 sum of square. The larger the IPCA scores, either negative or positive, the more specifically adapted a

genotype is to certain environments, smaller IPCA scores indicate a more stable genotype across environments.

### 2.3. Yield Stability Index (YSI)

A new approach known as yield stability index (YSI) is calculated by ranking the mean grain yield of genotypes (RY) across environments and rank of AMMI stability value (RASV). YSI incorporates both mean yield and stability in a single criterion as:

$$YSI = RASV + RY$$

Low value of this parameter shows stable genotypes with high mean yield.

## 3. Results

The results of combined analysis of variance (Table 1) showed highly significant differences for environments, genotypes and genotype  $\times$  environment interaction. As GE interaction was significant, it was possible to proceed and calculate phenotypic stability (Farshadfar and Sutka, 2003; Farshadfar and Sutka, 2006). Tukey, s test of additivity was not significant, while Bartlett test was significant.

Table 1: Combined analysis of variance for grain yield under different rainfed and irrigated conditions

| Source of variation | Degree of freedom | Mean square |
|---------------------|-------------------|-------------|
| Environment (E)     | 5                 | 16022.42**  |
| Error1              | 12                | 150.532     |
| Genotype (G)        | 10                | 3111.05**   |
| G $\times$ E        | 50                | 327.948**   |
| Error2              | 120               | 87.157      |
| Total               | 197               | -           |
| Bartlett test       | -                 | 73.55**     |

\*\* significant at 1% level of probability

Mean comparison revealed that the average grain yield of the genotypes ranged from 75.645 g for Sardary to 23.895 for disomic addition line 2R. The results of regression analysis (Table 2) indicated that the main effects of genotypes and GE interaction were relatively low, accounting for 24% and 13% of the total sum of squares (TSS) in the GE matrix, respectively, while the effect of environment was relatively high (63%). Using regression analysis (Table 2), the GE interaction was divided into two components: linear and deviation from regression. The linear component was not significant and accounted for 37% of the variability in the GE interaction. Using ANOVA, yield sum of square was partitioned into genotype, environment and GE interaction. GE interaction was further partitioned by principal component analysis (Table 3). The result of AMMI analysis indicated that 13% of total variability was justified by GE interaction, 63% by environment and 24% by genotypes.

Table 2: Regression analysis of Wheat-Rye disomic addition lines over different environments

| Source of variation       | Degree of freedom | Mean square           | TSS % |
|---------------------------|-------------------|-----------------------|-------|
| Genotype (G)              | 10                | 1036.15**             | 24    |
| Environment (E)           | 5                 | 5340.10**             | 63    |
| G $\times$ E              | 50                | 109.451**             | 13    |
| Source of variation       | Degree of freedom | Mean square           | GESS% |
| G $\times$ E(linear)      | 10                | 205.023 <sup>ns</sup> | 37    |
| Deviation from regression | 40                | 85.557                | 63    |
| Total                     | -                 | -                     | -     |
|                           | 65                | -                     | -     |

\*\* Significant at 1% level of probability, ns: non-significant

Table 3: Analysis of variance and the results of AMMI model in Wheat – Rye disomic addition lines

| Source           | Df | MS                    | TSS% |
|------------------|----|-----------------------|------|
| Genotype (G)     | 10 | 1036.15**             | 24   |
| Environment (E)  | 5  | 5340.10**             | 63   |
| G×E              | 50 | 109.451**             | 13   |
| Source           | DF | MS                    | TSS% |
| AMMI1            | 14 | 276.714**             | 71   |
| AMMI2            | 12 | 70.0112*              | 15   |
| AMMI3            | 10 | 41.898 <sup>ns</sup>  | 8    |
| AMMI4            | 8  | 35.2089 <sup>ns</sup> | 5    |
| Residual (noise) | 6  | 9.626                 | 1    |
| Total            | 65 | -                     | -    |

\*\* Significant at 1% level of probability, ns: non-significant

The results of AMMI analysis also revealed that AMMI1 (first interaction principal component axis (IPC1)) justified 71% of the interaction SS and AMMI2 (IPC2) explained 15% of GE interaction. The mean squares for AMMI1 and AMMI2 were significant and cumulatively accounted for 86% of total GE interaction. Therefore, the post-dictive evaluation using an F- test suggested that two AMMI1 and AMMI2 were significant for the model with 26 degree of freedom. In general AMMI2 model contained 99% of the treatment SS, while residual contained 1%. These results indicated that AMMI model fit the data well and justifies the use of AMMI2. IPCA scores of genotypes and environments took both positive and negative values (Tables 4, 5).

Table 4: First, second, mean, ASVi and YSLi of genotypes investigated

| Genotypes | IPCA1  | IPCA2   | Mean   | ASVi   | YSLi |
|-----------|--------|---------|--------|--------|------|
| 1R        | -1.085 | -1.047  | 42.112 | 26.203 | 13   |
| 2R        | 3.191  | 1.414   | 23.895 | 218.44 | 19   |
| 3R        | -3.447 | 2.187   | 42.731 | 257.34 | 15   |
| 4R        | 4.385  | 1.952   | 30.151 | 412.44 | 21   |
| 5R        | -0.905 | -0.525  | 45.219 | 17.68  | 8    |
| 6R        | 1.023  | -0.0363 | 34.635 | 22.22  | 14   |
| 7R        | -3.934 | 2.111   | 49.177 | 333.22 | 12   |
| CHS       | 0.024  | -2.169  | 43.511 | 4.72   | 7    |
| SAR       | -0.759 | -2.732  | 75.644 | 14.94  | 4    |
| RLO       | 1.353  | -1.047  | 41.032 | 39.97  | 15   |
| RIM       | 0.155  | 0.059   | 46.659 | 0.513  | 4    |

Table 5: IPCA1, IPCA2 and mean for environments

| Environments | IPCA1  | IPCA2  | mean   | ASVi   |
|--------------|--------|--------|--------|--------|
| A            | -2.699 | 0.120  | 82.328 | 154.81 |
| B            | 2.614  | -4.234 | 38.239 | 469.9  |
| C            | 1.820  | 0.150  | 36.142 | 70.42  |
| D            | -5.953 | -0.215 | 53.447 | 153.24 |
| E            | 1.625  | 1.029  | 22.144 | 58.17  |
| F            | 2.593  | 3.149  | 26.662 | 152.79 |

According to the ASV values which quantify the results of AMMI model, genotypes 8, 11 and disomic addition line, 5R showed the most stability. Biplot analysis and ordination techniques revealed highly significant differences for IPCA1 (AMMI1) and IPCA2 (AMMI2) which cumulatively justified 86% of variability in the GE interaction. Biplot analysis (Fig 1) also displayed that genotypes 1, 5, 8 and 11 were toward the center of biplot, therefore have zero interaction. As genotypes 9, 8, 5 and 11 have mean yield over the grand mean they are considered as stable genotypes.

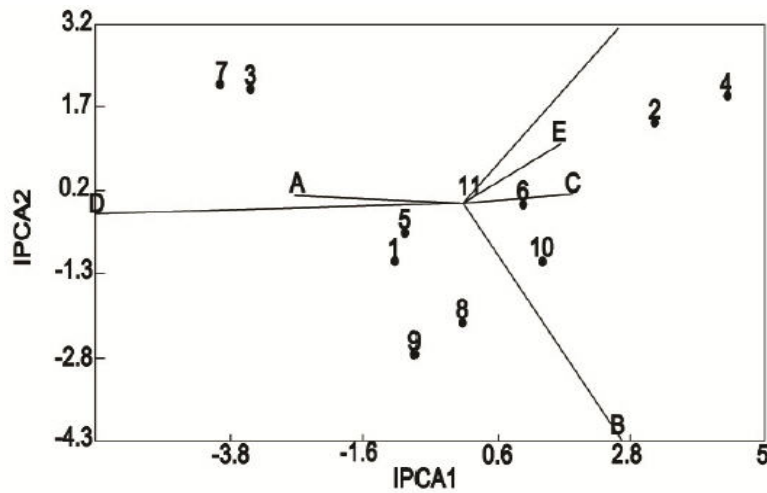


Figure 1: Interaction biplot for AMM2 model

As AMMI2 revealed the least RMSPD (root mean square predictive difference), therefore recommendation must be based on this model (Crossa et al., 1990; Wade et al., 1995; Farshadfar and Sutka, 2006).

As ASV take into account both IPCA1 and IPCA2 that justify most of the variation of GE interaction, therefore the rank of ASV and mean yield (RY) were incorporated in a single selection index namely: yield stability index (YSI). The least YSI is considered as the most stable with high mean yield. Based on YSI the most desirable genotypes were RIM, SAR followed by CHS, while the best disomic addition line was 5R.

#### 4. Discussion

Significant results of combined analysis of variance indicated the effect of environment in the GE interaction, genetic variability between the genotypes and possible chromosomal localization of the genes controlling adaptation (Farshadfar and Sutka, 2003; Farshadfar and Sutka, 2006). Tukey, s test of additivity was not significant, leading to the conclusion that the effects are additive, hence the conditions required for analysis of variance are provided (Snedecor and Cochran, 1989).

Bartlett test was significant exhibiting the heterogeneity of error variance, but as transformation of data caused missing some information and incorrect decision, hence no transformation exerted on the actual data (Hugh and Gauch, 1988). The linear component of GE interaction in the regression analysis was not significant indicating the homogeneity of the regression coefficients and the disadvantage of regression analysis for stability discrimination (Wade et al., 1995). As a general rule the effectiveness of regression analysis is when 50% of the total sum of squares is accounted for by linear GE interaction (Hayward et al., 1993).

##### 4.1. AMMI Model and Pattern Analysis

In AMMI model, principal component analysis is based on the matrix of deviation from additivity or residual, while pattern analysis employs both classification and ordination techniques. In this respect, both the results of AMMI analysis, the genotype and the environment will be grouped based on their similar responses (Gauch, 1992; Wade et al., 1995; Pourdard and Mohammadi, 2008).

Adjusted grain yield can be obtained by AMMI1 and AMMI2 using the formula:  $\bar{Y}_{i0} + \bar{Y}_{0j} - \bar{Y}_{00}$  where  $\bar{Y}_{i0}$  is the mean yield of genotype,  $\bar{Y}_{0j}$  = mean yield of environment and  $\bar{Y}_{00}$  = grand mean for each environment and is used as a selection criterion in breeding programs. A large contribution of environment showed that environments were diverse, with large differences among environmental means causing most of the variation in grain yield. The magnitude of the GE interaction was almost half than that of the genotypes, indicated that there were fine differences in the genotypic response across environments. In general the results of AMMI analysis indicated that AMMI model fit the data well and justifies the use of AMMI2. It is to be mentioned that the importance of AMMI model is in reduction of the noise even if principal component do not cover much of the GESS (Gauch and Zobel, 1989; Gauch, 1992).

## 4.2. IPCA, Crossover and Non-Crossover Interaction

IPCA scores of genotype and environment took both positive and negative values (Tables 4, 5). Consequently, a genotype that has large positive IPCA score with some environments most have negative interaction with some other environments. Thus, these scores presented a disproportionate genotype response (Yan and Hunt, 2001; Mohammadi et al., 2007a), which was the major source of variation for any crossover (qualitative) interaction. This disproportionate genotype response is referred to as crossover GE interaction for convenience. Diversely, scores with the some sign or near zero represent a non – crossover (quantitative) GE interaction or a proportionate genotype response (Mohammadi et al., 2007b; Mohammadi and Amri, 2008).

## 4.3. AMMI Stability Value (ASV)

The AMMI model does not provide a quantitative stability measure, such a measure is essential in order to quantify and rank genotypes according to their yield stability, that is why ASV is proposed by Purchase et al. (2000). In fact ASV is the distance from zero in a two dimensional scattergram of IPCA1 ( interaction principal component analysis axis 1) scores against IPCA2 scores. Since The IPCA1 score contributes more to GE sum of squares, it has to be weighted by the proportional difference between IPCA1 and IPCA2 scores to compensate for the relative contribution of IPCA1 and IPCA2 to total GE sum of squares. The distance from zero is then determined using the theorem of Pythagoras (Purchase et al., 2000). In ASV method a genotype with least ASV score is the most stable, accordingly, genotypes RIM and CS were the most stable and among disomic addition lines, genotype 5R is the most stable one. It can be concluded that chromosome 5R carry most of the genes controlling adaptation in Rye . Chromosomes 2R, 3R, 4R and 7R were undesirable for improvement of adaptation. Stability of disomic addition line 5R indicated that most of the QTLs controlling phenotypic stability are located on chromosome 5R. Genotypes 2R and 4R showed specific adaptability with environments E and F, genotypes 3R, 7R and 9 with environments A and D, while genotypes 6E and 10 with environments B and C. In biplot analysis genotypes are judged in grouping form and therefore save time and precision in interpretation and selection (Wade et al., 1995; Alagarswamy and Chandra, 1998), therefore have general adaptability with different mean grain yield.

## 4.4. Yield Stability Index (YSI)

Stability perse should however not be the only parameter for selection, because the most stable genotypes would not necessarily give the best yield performance (Mohammadi et al., 2007a), hence there is a need for approaches that incorporate both mean yield and stability in a single criterion, that's why Kang (1991, 1993) introduced three selection criteria for simultaneous selection of yield and stability entitled: rank sum (RSM), modified rank–sum (MRSM) and the statistics yield – stability (Ysi). In this regard, as ASV take into account both IPCA1 and IPCA2 that justify most of the variation of GE interaction, therefore the rank of ASV and mean yield (RY) are incorporated in a single selection index namely: yield stability index (YSI). The least YSI is considered as the most stable with high mean yield. Based on YSI the most desirable genotypes are RIM, SAR followed by CHS, while the best disomic addition line is 5R, therefor it can be concluded that most of the genes controlling both yield and yield stability in Rye are located on chromosome 5R. It is therefore suggested to use chromosome 5R for QTL mapping using molecular markers and transferring into wheat genetic background for enhancement of yield and yield stability over rainfed and irrigated conditions. The importance of chromosome 5R for improvement of drought tolerance was also reported by Farshadfar et al. (2003).

## 5. References

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