

Variation analysis of wheat F₃ lines produced by crossing between Azar2 and 87-Zhong291 cultivars using RAPD method in drought stress condition

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Abstract

In order to analyze variation and find an efficient selection approach, we used crossing results between two wheat cultivars, Azar2 and 87-Zhong291. F₃ plants (374 lines) and four check cultivars planted in the form of augmented experiment. For germination, seeds were irrigated once. After using multiple statistics methods such as cluster analysis, 79 different lines were selected and used for RAPD analysis with UBC3 primer that had produced six polymorphic bands (447 fragments in total). Jaccard's coefficient for parents was low (0.17). Therefore, Azar-2 and 87-Zhong291 had extreme differences with each other. For the analysis of relationship between polymorphic bands and morphological characters, logistic regression was used. The majority of characters were not related to the bands but some of morphological characters, such as biological yield, plant height, main spike weight, 1000 grain weight and peduncle length showed a negative relation with some of polymorphic bands. Among them, seed number/spike trait had a positive relationship with a 600bp band. This band could be used as a selection marker for seed number/spike trait.

Key words: Wheat, Drought tolerance, logistic regression, segregated plants, RAPD.

Introduction

Presumably, wheat is one of first crops, cultivated by human and from the nutrition point of view, it is the most important crop plant as it supplies 20% of calories of human in the world. In addition, in Iran wheat is the main source that provides calories and protein requirement of country population, as it consists of 75% protein and 65% of calories, each person needs daily (Yazdi-Samadi and Abd-Mishani 1996; Khodabandeh 1995; Abd-Mishani and Jafari Shabestari 1998; Karimi 1992 and Anonymous 2002-03). The cultivation area of wheat in Iran is estimated to be around 6.41 million hectares, which 37.42% is irrigated and 62.58% is in dry conditions. Watered wheat yield is 3629 and dry wheat yield is 1181 kg/ha in Iran (Anonymous 2002-03). Considering the high percentage of wheat cultivation in dry conditions in Iran, and limited water, any effort for increasing drought tolerance in wheat cultivars can be an important step forward for food supply. To make improve drought tolerance in wheat by genetic diversity, there is a need to consider useful methods that can be fast able to evaluate plant responses in the critical developmental stages and large populations could be screened using small samples from individuals. Today, detection of desirable traits and use of marker aided selection by the determination of their

linkage with agronomically important traits (qualitative and quantitative) has become possible. This approach provides the possibility of rapid and accurate selection of desirable genotypes in the preliminary growth stages and make the lengthy breeding period shorter (Yazdi-Samadi and Abd-Mishani 1996; Khodabandeh 1995; Abd-Mishani and Jafari Shabestari 1998; Karimi 1992 and Anonymous 2002-03). He et al. (1992), Devos and Gale (1992) and Joshi and Nguyen (1993) observed polymorphism between wheat genotypes using RAPD markers. He et al. (1992) used 65 using primers, out of which, 38% were able to distinguish polymorphism between So852 spring and Clark wintry varieties with repeatable results. They also found RAPD markers useful in the investigation of polymorphism between recombinant inbred populations

Tar et al. (2002) used 81 RAPD primers in order to find a suitable marker for yellow rust resistance gene (*Lr29*). One of primers showed a different banding pattern and was linked to the resistance gene for yellow rust. Genetic linkage of this marker with *Lr29* gene was further investigated on segregating F_2 plants (derived from crossing between a resistance line and a susceptible line). This marker showed a close linkage with *Lr29* gene. The amplified band present only in the resistant lines was cloned and sequenced. The specific primers (SCAR) amplified the desired band only in the resistance lines.

To obtain an appropriate selection index, results obtained from morphological traits and molecular markers could be combined. This research was designed to study a) different genotypes from segregating plants (F_3), produced by crossing Azar2 and 87-Zhong291 varieties in drought stress conditions and assess their agronomical traits, b) genetic variation in the segregating plants by agronomical traits and RAPD marker and c) discover any possible relationship between RAPD markers and agronomical traits.

Materials and Methods

In order to analyze variation in F_3 plants (374 lines), crossing results between Azar2 (drought tolerant) and 87-Zhong291 (drought susceptible) were used. This cross designed in a form of augmented experiment with four control cultivars (Azar-2, 87-Zhong291, Kharchia and Superseri#2). Seeds were planted in cereals section farm of seed and plantlet certification institute of Karaj. The control varieties planted in RCBD design with 7 replications. For germination of seeds, the farm was irrigated just once. During the growth season and its end various morphological characters were measured. For adjusting lines, data from control cultivars were used and adjusted lines were compared together. Finally, the lines were clustered by UPGMA method and SPSS software. By considering the results of this experiment, 79 different lines were selected for molecular studies.

The seedlings grown from the seeds of 79 selected lines and their related parents were used for DNA extraction. Fourteen days after germination, DNA was extracted from leaves, according to a CTAB mini-preparation method (Aldrich and Cullis 1993). UBC3 primer (5'-CCT GGG CTT A-3') was purchased from Cina Gene Company (Tehran, Iran).

Polymerase Chain Reaction (Williams et al. 1990) was prepared with the following components: 2.5 μ l PCR buffer(1 x), 2.5 mM $MgCl_2$, 10 μ M each of the dNTPs, one unit of Taq DNA polymerase, 15 ng of primer, 20 ng of template DNA and 11.05 μ l ddh₂O to the final volume of 25 μ l.

Thermo cycling was programmed as: initial denaturation for 2min at 94 °C, 40 cycles of 1 min at 92 °C, 1 min for primer annealing to singular strand DNA at 35 °C and 2 min at 72 °C for extension and a final extension of 5min at 72 °C. After

complete the cycles, samples were kept at -20 °C.

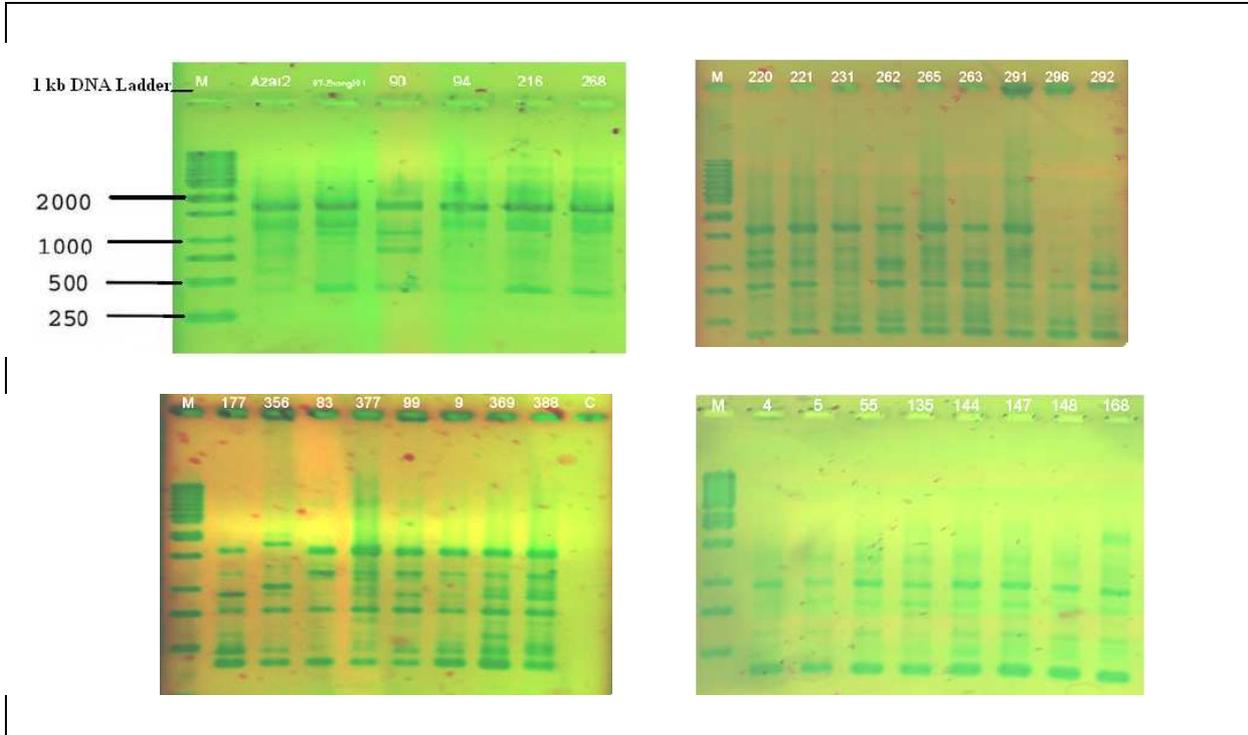


Fig. 1. Produced DNA fragments by UBC3 primer in Azar2, 87-Zhong291 varieties and some F₃ generation lines.

Results

Based on the morphological traits of the 374-planted lines, 79 lines were selected. The UBC3 primer totally produced 447 fragments. In the genotypes 6 polymorphic bands with the size range of 600 to 1750 bp were detected (Fig. 1). Similarity of the lines with each other and with either of the parents was evaluated by using Jaccard's similarity coefficient. This coefficient varies between zero and one. The closer it gets to 1, similarity between individuals becomes higher. For example, line 4 had the maximum similarity (1.0) with lines 5, 71, 144 and 148, and line 47 had the maximum similarity (1.0) with lines 69, 133, 174 and 208. But with approach, as the numeral scale of this coefficient gets close to zero, similarity between lines will become lower. For example, line 4 had the

maximum disparity (0.17) or the least similarity with lines 33, 60, 63 and 277. So if one has to choose parents for the creation of diversity in the next generation,

one should use crosses involving the most distant individuals. In addition, Jaccard's coefficient calculated between Azar2 and 87-Zhong 291 varieties was 0.17. This shows the correct selection of these two varieties as parents for the creation of segregating generations.

The relationship between morphological traits and polymorphic bands

To investigate any relationship between the presence of a 600 bp polymorphic band and morphological traits, logistic regression was used. In this analysis, statistical characteristics with χ^2

distribution were used. By performing the forward stepwise regression, the independent variables entered the model systematically. In this order, in the first stage, total height trait, in the second stage, the number of grains in spikes and in the next stage, biological yield entered the model. Negative coefficient of total height and biological yield variables showed that with increasing the trait; probability of the presence of the response variable (band) will decrease. Positive coefficient of the number of grains/spike trait showed that with increasing this trait; the probability of presence of response variable (band) will increase. Base on the constant value and B coefficient, the logistic regression optimum equation were obtained as below:

$$\text{Logarithmic primacy} = 5.979 - 0.073 (\text{total height}) - 0.163 (\text{biological yield}) + 0.098 (\text{no. of grains/spike})$$

With logistic regression calculation between presence or absence of the 800bp band with other traits, only the weight of main spike trait with negative coefficient (-0.069) entered the model as:

$$\text{Logarithmic primacy} = 2.492 - 0.069 (\text{weight of main spike})$$

Since the B coefficient is negative, it could be interpreted as a negative relation between the weight of main spike and existence of the 800bp band. However, considering the significance level (0.761) this relationship is not shown significant statistically.

The relation between morphological traits and the presence or non-existence of a 900bp band was studied. A highly significant ($\alpha = 1\%$) negative correlation was found between the increase in the 1000 kernel weight and the presence of this band. In other words, by increasing 1000 kernel weight, the probability of observing the 900bp band decreases.

$$\text{Logarithmic primacy} = 5.185 - 1.073 (1000 \text{ K.W.})$$

The relation between morphological traits and the presence or absence of a 1000bp band was studied. There was a significant ($\alpha = 5\%$) negative correlation between the increase of 1000 kernel weight and the presence of this band. In other words, by increasing 1000 kernel weight the probability of observing the 1000bp band decreases.

$$\text{Logarithmic primacy} = 2.522 - 0.573 (1000 \text{ K.W.})$$

In the study of relation between 1150bp band and the morphologic traits, none of traits entered the model. Therefore, there isn't any relation between this band and the morphologic traits.

The relation between the morphologic traits and the presence or absence of 1750bp band was investigated. A significant ($\alpha = 5\%$) negative correlation was discovered between the length of peduncle and the presence this band. In other words by increasing peduncle length, the probability of observing the 1750bp band decreases.

$$\text{Logarithmic primacy} = 8.060 - 0.171 (\text{length of peduncle})$$

Generally speaking, it could be said that the majority of the observed polymorphic bands, had no correlations with morphological traits, or showed a negative correlation. Nevertheless, the 600bp band showed a positive correlation with the number of grains/spike trait. Number of grains/spike trait is one of the most important parts of yield in wheat. Genotypes, which show stability for this trait, often will show better tolerance in drought conditions. So it could be used as a marker for the selection of varieties bearing more grains in spike.

Finally, the observations of this research showed that RAPD markers are quick and

inexpensive for genetic variation studies in wheat and also it is useful for the selection of parental lines to be crossed for superior hybrid production programmes. In addition, according to the Jaccard's similarity, high yielding lines could be selected in F₃ populations. Therefore, selection could be performed to establish next generations and get close to pure lines.

Discussion

If parents are more distant to each other, the possibility of creation of superior individuals (heterosis) will be more. In this study, we used parents with extreme reactions to drought conditions. The results of morphological analysis and Jaccard's similarity coefficient (0.17) confirmed this fact. Several populations were resulted from the crossing. These populations could be used for many objects such as genetic analysis and QTL mapping as well as applied breeding processes for drought resistance and other appropriate characters. There are some methods for determining relationships between morphological traits and molecular markers, but in this study, we showed that logistic regression could be a reliable and simple method for this kind of studies. In the method, there is not any limitation on the population size and the number of primers. In this study, we used 79 individuals with just one primer for RAPD markers. Using this method, we analyzed the relation between independent quantitative and binominal variables. Most of the characters were not related with the bands produced by PCR reaction. Some characters, such as biological yield, plant height, main spike weight, 1000-grain weight and peduncle length were negatively related with some of polymorphic bands. Among them, seed number/spike trait was positively related to a 600bp band. The use of is suggested as a selection marker for seed number/spike trait in drought stress conditions.

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