Using single morphological and RAPD molecular markers to screen for quantitative traits in an F_2 segregating generation of wheat (*Ttiticum aestivum L.*)

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ABSTRACT
The F_2 individuals were classified into 2 groups based on presence or absence of the bands amplified by the primers which showed polymorphism in parents and F_2 and based on two qualitative traits (including hairy glumes and growth habit at the early stages), separately. The results obtained by comparing the means, using t-test, revealed that subpopulations classified by the presence or absence of the polymorphic band amplified using OPM35 were significantly different for heading date. On the other hand, subpopulations classified by presence or absence of bands amplified using OPG12 were significantly different for days to maturity. No significant difference was found between the subpopulations formed by presence or absence of the bands of the either primers for grain weight. Classifying the F_2 individuals, based on the form of growth at the early stages, resulted to 2 subpopulations; one having erect and the other rosette forms of growth. These two subpopulations were significantly different for grain weight, days to heading and days to maturity. Conversely, 2 subpopulations identified by having or lacking hairy glumes were significantly different for none of the traits. Correlation coefficient analysis indicated that hairy glume, OPG12 and OPM35 were correlated with none of the other characters. On the other hand, rosette shape was significantly correlated with grain weight, days to heading, days to maturity and harvest index. In addition, grain weight per plant correlated positively to Harvest Index (HI) and negatively to Days to Heading (DH).

INTRODUCTION
Identification of molecular bands or morphological markers associated with grain weight is the most important step in selecting genotypes having higher yield at the early stages of growth. This study was designed to investigate the characteristics of agronomic values in different groups (clustered on the basis of the presence or absence of RAPD and morphological markers) in an attempt to find a possible link between molecular and morphological markers with quantitative traits. This study was also aimed to determine the relationship between agronomic traits and grain weight in an F_2 generation.

MATERIAL AND METHODS
The RAPD profiles were analyzed based on the presence (1) or absence (0) of individual RAPD bands. Two molecular bands, amplified by primers OPM35 and OPG12, respectively, indicated polymorphism in parents as well as in the F_2 populations (Fig 1). Knowing these results, the F_2 individuals were classified into 2 groups based on (a) the presence or absence of the bands amplified by the primers, or (b) the presence or absence of qualitative traits (including hairy glumes and rosette shape), separately. With this explanation, it is clear that 4 visual grouping systems were provided. In each case, the quantitative characters of individuals in the two groups were then recorded and a t-test was performed to determine if the differences observed between the means of the two groups were significant for grain weight and other quantitative traits. In addition, Pearson correlation analysis was also performed in order to simply identify possible phenotypic links between the characters, as well as comparing the results obtained using correlation analysis with those obtained using the t-test. For performing correlation analysis between quantitative and qualitative characters, the latter characters were coded as 0 and 1 (having the character 1 and lacking the character 0).

RESULTS AND DISCUSSIONS
A simple approach, unlike any methods observed in the literature, was used to cluster the F_2 population. In this method single markers were used to differentiate individuals within the F_2 population. By contrast, almost all other investigators have used multivariate cluster analysis to classify individuals into different groups. Clustering F_2 populations based on the genetic distance calculated using different polymorphism RAPD markers may lead to better results when differentiating between individuals for quantitative traits, rather than presence or absence of single markers. **A** study carried out by Liu et al (1999) used fifty-four RAPD markers generated by six primers to study their potential value in distinguishing between parents with different characteristics and predicting the yield performance of hybrids produced from these parents. Based on the above results, they concluded that it is possible to differentiate wheat lines, with different performance, using RAPD markers. The latter method was not a case for the present study because there were only two polymorphic markers, not sufficient to create the genetic distance matrix. The results of the present study revealed that differentiating wheat genotypes for quantitative characters, in the segregating generation, is also possible by selecting and grouping the individuals based on the presence or absence of single markers followed by t-test analysis, for comparing the means of different groups even when there are only a few polymorphism markers. This can be useful because there is no guarantee of finding the high number of polymorphism markers

required for calculating genetic distance matrices. Application of the above mentioned classification method, in which presence or absence of a single marker was considered, can be suitable, at least when there is only a few polymorphic markers. In addition, the classification based on this method is much easier than performing multivariate analyses, such as cluster or discrimination methods.

Comparison of the results obtained by the t-test with those obtained with correlation analysis revealed that the two statistical analyses did not perform in a similar way for all the characters. For instance, t-test analysis revealed a significant difference between the subpopulation having the polymorphic band amplified by using OPM35 and that lacking the band for days to heading. On the other hand, the correlation coefficient between this band and days to heading was not significant. These results imply that correlation analysis (data not shown) does not support the t-test findings. The reason for this inconsistency may relate to the assumption needed for parametric analyses, such as correlation coefficient. When using distinct numeric 0 and 1 as one set of data, the statistical assumption for correlation analysis (means normally distributed) (Steel and Torrie 1976) is not achieved. Thus, it seems that clustering individuals into two groups based on the absence or presence of a particular marker and then performing t-test to compare the means of the two groups having homogenous quantitative data, is more compatible with statistical theory and, possibly, more reliable than calculating the correlation between two heterogeneous sets of data.

The molecular marker, amplified by OPM35, was able to differentiate F_2 individuals for heading date. This character is one of the most important traits for wheat performance and improvement because it affects the adaptability of the crop to environmental conditions, including water-stress (Law 1998). The genetic control of heading date is complex with many genes responsible for expression, from which 3 main groups can be identified - photoperiod response, vernalization response and earliness genes *per se*. This complexity then implies that direct selection for the character is less likely to be successful and that using molecular markers is likely to result in a higher gain of selection.

Vernalization requirement is the sensitivity of plants to cold treatment for accelerating spike primordia development (Kato *et al.* 2001). Semi-winter varieties such as the variety used in the present study (FM36) remain in a rosette shape for a period up to 30 days from spring planting where they do not meet vernalization requirements. In these situations, vernalization sensitive genotypes reach the stage of stem elongation after delays. This makes it possible to identify the response of the plants when they are grown without any artificial cold treatments. Thus using a rosette shape as a morphological marker in order to differentiate the F_2 individuals (data not shown) reflects, in fact the effects of vernalization response. Vernalization responses of wheat cultivars play an important role in adaptation of these cultivars to environmental conditions. Although the effect of vernalization is generally to promote earlier floral development, the effect of vernalization on the heading date and morphological characters is dependent on genotype and environment (Kato *et al.* 2001). Vernalization requirement is related to drought and heat tolerance in the regions in which plants are to be grown under limited water supply or high temperatures. In this situation, if the temperature is not low enough to satisfy vernalization, plants produce massive vegetative tissues in the spring and consequently use a high proportion of the water supply before starting the reproductive stage. Even if water supply is not limited, lack of vernalization postpones anthesis and can cause problems, such as those caused by high temperatures during the grain-filling period. Such a phenomenon happened in the present study causing a significant reduction in grain weight of the genotypes sensitive to vernalization.

To sum up, the results of the present study revealed that using single molecular markers to screen for quantitative traits is possible and the experiments undertaken using molecular markers can be analyzed even if only one polymorphic molecular band is amplified. However investigating the quantitative characters in the classified groups at the F_3 generation could reveal further useful information for crucial speculation regarding the links between the phenotypic markers and quantitative characters.

Table 1: The differences observed between the subpopulations classified based on the presence or absence of the polymorphic band amplified using OPM35 for the quantitative traits under study

Trait	Presence	Absence	Difference	P value
Days to heading	79.62	86.77	-7.15	0.02
Spike length(cm)	8.18	7.62	0.56	0.31
Grain yield/plant(gr)	3.22	2.16	1.06	0.20
Harvest index	0.18	0.16	0.02	0.46
Days to maturity	111.48	113.42	-1.96	0.22

Table 2: The differences observed between the subpopulations classified based on presence or absence of OPG12 for the quantitative traits under study

Trait	Presence	Absence	Difference	P value
Days to heading	79.02	83.76	-4.74	0.06
Spike length(cm)	8.23	7.87	0.36	0.37
Grain yield/plant(gr)	3.54	2.55	0.99	0.15
Harvest index	0.19	0.17	0.02	0.38
Days to maturity	110.78	116.6	-5.82	0.01

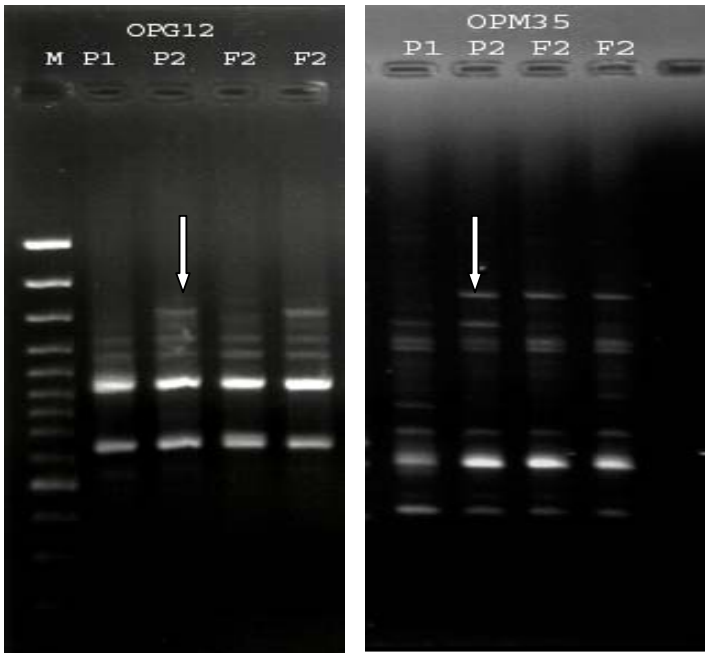

Figure 1: Bands showing polymorphism between parents and F_2 individuals (only two plants of F_2 are shown)

REFERENCES

1. Kato K, Minura H & Sawada S (2000) Maapping QTLs controlling grain yield and its components on choromosomes 5A of wheat. *Theoretical and Applied Genetetics* **101**, 1114-1121.
2. Law C N **(**1998**)** Genetic control of flowering in wheat-a personal view. In: Proceedings of 10th EWAC Meeting. (Eds. C. Ceoloni & A. J. Worland) pp. 46-52. Viterbo.
3. Liu ZQY, Pei Z & Pu J (1999) Relationship between hybrid performance and genetic diversity based on RAPD markers in wheat, *Triticum aestivum* L. *Plant Breeding* **118** , 119–123.
4. Steel RGD. & Torrie JH (1976) Introduction to Statistics. McGrow-Hill. New York .