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Evaluation of Pre-harvest Sprouting Resistance in Durum Wheat Germplasm

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Abstract: Pre-harvest sprouting seriously reduces durum grain quality and is considered as an important grading criterion in all market classes of wheat (*Triticum* sp.). In northern Iran, untimely rain and humid conditions at harvest time cause sprouting of kernels in spike. There are few researches in the literature on pre-harvest sprouting and related traits in durum wheat (*Triticum turgidum* L. var. *durum*). A two years study (2001 and 2002) was conducted to evaluate the genetic variation of sprouting resistance and related trait in the Iranian germplasm of durum wheat accessions. In 2001, a total of 532 accessions were planted under field conditions. Unreplicated samples of 10 spikes from each genotype were evaluated for spike sprouting and falling number under laboratory conditions. Using two screening methods, the 2001 study indicated that durum accessions had a similar range in sprouting score and sprouting index to the wheat check cultivars. The sprouting score for durum accessions ranged from zero to 10. Twenty-eight of selected accessions with high level of sprouting resistance were re-evaluated in a replicated study in the second year. Sprouting score and sprouting index measured in 2002 were correlated with those of 2001. According to the results of this study, there is considerable genetic variation in sprouting resistance in durum germplasm that can be exploited by breeding programs.

Key words: Pre-harvest sprouting, sprouting score, sprouting index, durum wheat

INTRODUCTION

Pre-harvest rains on cereals coupled with favorable temperatures create conditions suitable for cereal grains to germinate on the mother plant. Pre-harvest sprouting can be a serious periodic problem in the production of high quality durum genotypes (*Triticum turgidum* L. sp. *durum*) used for pasta manufacture (Nagao, 1996). When grain is exposed to rainfall, imbibition occurs and germination starts immediately. Amylase enzymes build up rapidly gives the kernel a bleached and mealy texture (Derera, 1989). The damaged kernels have low test weight and high paste viscosity. Grant *et al.* (1993) reported that for semolina and spaghetti, sprouting in durum wheat caused higher cooking losses and a decrease in firmness.

In northern Iran, a low level of tolerance to pre-harvest sprouting is undesirable because, occasionally, untimely rains and/or prolonged damp conditions at harvest time cause sprouting of kernels in the spike. Pre-harvest sprouting susceptibility in cereals is a consequence of low grain dormancy before harvest (Rodriguez *et al.*, 2001). It is generally accepted that the long term solution to the pre-harvest sprouting problem lies in the development of cultivars which are able to tolerate or resist the damaging effects of rain during the period between maturity and the completion of harvest. Transfer of sprouting resistance from common to durum wheats would be tedious and, in the case of transfer from red common wheats, potentially unsuccessful (Soper *et al.*, 1989).

The present study sought to assess genetic variation for pre-harvest sprouting resistance and related trait in Iranian durum accessions in comparison to that available in common wheat, thereby seeking potential sources of sprouting resistance in existing genotypes or germplasm. Moreover, two screening methods will be evaluated for their validity and reliability when dealing with a large number of genotypes.

MATERIALS AND METHODS

Five hundred and thirty-two Iranian durum genotypes from the cereal germplasm collection, College of Agriculture, University of Tehran were selected. Three hexaploid cultivars (one from Canada and two from Australia), four durum cultivars (two from Canada and two from USDA germplasm) and two commercial durum cultivars (Stoke and Yavares) were used as controls. These control cultivars represent various resistances in sprouting (Table 1). All genotypes were planted in mid

Table 1: Durum and common wheat genotypes used as sprouting resistance checks in 2001 and 2002

Genotype	Source	Sprouting phenotype
RL4137	Canada	Resistance
AUS1293	Australia	Resistance
AUS1408	Australia	Resistance
Stewart	Canada	Moderately resistance
Kyle	Canada	Moderately resistance
93-951	USDA germplasm	Moderately resistance
93-955	USDA germplasm	Moderately resistance

October, 2001, into a silty clay soil at the University of Tehran's Research Farm in Karaj. Plots consisted of two 3 m long rows spaced 0.25 m apart. The plots were randomized but not replicated. Plots were irrigated eight times between seeding date and harvest. The following traits were measured for all the genotypes: days to 50% spike emergence, days to anthesis and days to physiological maturity, which coincides with loss of green color from the flag leaf and glumes (Hanft and Wych, 1982). At Zadoks' Growth Stage 92 (ZGS92) (Zadoks *et al*., 1974), fifty upper-canopy spikes per plot were harvested. The spikes were held at room temperature for seven days and then placed in a freezer at -20°C to maintain dormancy (Mares, 1983).

For sprouting evaluation, ten intact heads per plot were immersed in distilled water for 3 h. To establish a humid condition, upon removal, the spike bundles were placed in plastic bags and kept in a germinator (Grouk Company, Iran) for seven days. The photoperiod inside the chamber was set for 16 h light and 8 h dark. The chamber was maintained at 25°C and 87% humidity in the light and 15°C and 70% humidity in the dark. After seven days, the heads were threshed by hand and the number of germinated and ungerminated kernels counted. Individual spikes were rated based on two screening methods, namely, sprouting score and sprouting index. Sprouting score is the number of spikes out of 10 showing visible sprouts (Clarke *et al*., 1994). Sprouting index was determined using the following formula (Hucl, 1995): $SPRT=(\Sigma 0\text{-}5)/n\times 5)\times 100$, where n = number of spikes rated and individual spikes were rated on a scale of 0 to 5, where 0=no visible sprouts and 5=100% of the primary and secondary floret grains sprouted.

A subsample of 28 accessions with high levels of sprouting resistance in the 2001 test was selected for further evaluation. These together with the similar checks, were grown in three-replicate randomized complete block trial in 2002. Plot configuration was the same as in 2001. Spike samples for sprouting evaluation test were collected and tested as in 2001.

The Falling Number (FN) method was used to determine the alpha-amylase activity in flour. Three replications of 10 g of grain was ground in a Falling Number 3100 mill (Perten Instruments, Sweden). Distilled water (25 mL) was poured into a viscometer tube with a dispenser. Seven gram of the sample were weighed and put into the tube. Sample and water were mixed by vigorously shaking the tube in to obtain a homogeneous suspension. The viscometer tube with a stirrer inserted was put into a boiling waterbath and the machine was started. The total time, in seconds, from the start-up of the apparatus until the stirrer has fallen a measured distance was registered by the Falling Number 1500 equipment (Perten Instruments, Sweden).

RESULTS AND DISCUSSION

The accessions used in the present study showed a maturity range of about 47 days. Genotypes DR-82, DR-83 and DR-86 were the latest maturing (216 days) while DR-416 the earliest (169 days). However, sprouting score and sprouting index were not correlated significantly (r = -0.03 and -0.02, n = 541) with maturity in 2001. Likewise, low correlations were detected between days to spike emergence (r = 0.0.6 and 0.11, n = 541) and days to anthesis (r = 0.04 and 0.09, n = 541) with sprouting score and sprouting index, respectively. Earlier reports in the literature suggest that sprouting resistance in wheat is associated with delayed maturity. Based on a study of 11 cultivars, Derera *et al*. (1977) reported a moderate (-0.48 to -0.57) correlation between days to anthesis and the degrees of sprouting and concluded that selection for increased sprouting resistance without selection for earliness could lead to later maturity. DePauw and McCaig (1991) reported no (-0.16 to -0.30, ns) correlations between sprouting and spike emergence in a study of 26 spring wheat cultivars. Hucl (1995) found that selection for earliness in segregating populations of bread wheat may result in reduced sprouting resistance. Conversely, selection for increased sprouting resistance can lead to delayed maturity. However, this association was not consistent across all populations. He concluded that selection for early maturity did not have a consistent effect on sprouting tolerance. Tavakkol-Afshari and Hucl (2002) reported no significant correlation between sprouting resistance and days to spike emergence or days to physiological maturity in Canadian and USDA durum accessions

There is considerable genetic diversity in the expression of sprouting resistance among durum genotypes. Using Clark's method, the distribution of sprouting scores of durum genotypes tested in 2001 relative to that of the controls suggests that the range in pre-harvest sprouting resistance of durum is similar to that of hexaploid and tetraploid wheat controls (Fig. 1). Sprouting scores of both the checks and durum genotypes ranged from low to high sprouting resistance.

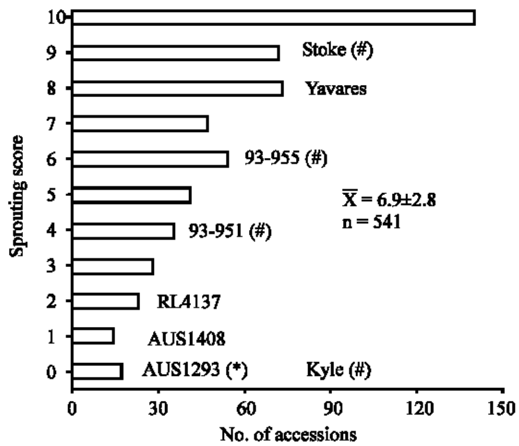

Fig. 1: Frequency distribution for sprouting score (number of spikes out of 10 showing visible sprouts) of durum genotypes tested in 2001 compared with that of durum (#) and common (*) wheat control

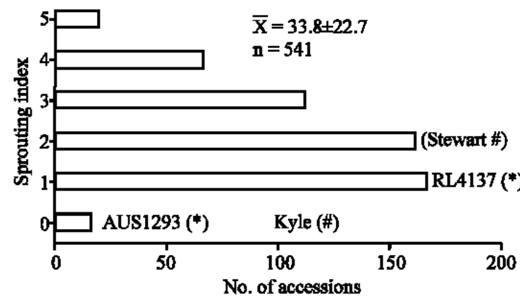

Fig. 2: Frequency distribution for sprouting index (0 = no visible sprouts and 5 = 100% of primary and secondary floret grains sprouted) of durum accessions tested in 2001 compared with that of durum (#) and common wheat checks (*)

Of the durum checks, cultivar Kyle and 93-951 had the lowest (zero and four, respectively) and cultivar Stoke had the highest (nine) sprouting score. All hexaploid controls including RL4137, AUS1408 and AUS1293 exhibited high resistance to pre-harvest sprouting which is consistent with the observations of Hare *et al.* (1988) and Clarke *et al.* (1994). A number of durum genotypes exhibiting high levels of sprouting resistance. DR-103 and DR 114 with sprouting score of zero were among the resistant genotypes. However, most genotypes had low to moderate level of pre-harvest sprouting resistance.

Similar to the sprouting score, a wide range of responses was found among genotypes for sprouting index in 2001. Sprouting index of durum accessions exhibited a similar range of resistance to check cultivars (Fig. 2). Among hexaploid genotypes, AUS1293 and RL4137 with sprouting indices of zero and one, respectively, exhibited high levels of sprouting resistance. Among durum checks Kyle was the most resistance one. Using this screening method, a number of promising genotypes with high levels of sprouting resistance were detected. Sprouting score was highly correlated with sprouting index ($r = 0.82$, $n = 541$) in 2001 indicating both screening methods could efficiently detect sprouting resistant genotypes.

Analysis of variance detected significant differences among accessions for sprouting score in 2002 (data not shown). However for sprouting index the differences among accessions were not significant. The 28 durum genotypes with low sprouting scores and sprouting indices for 2001 and 2002 are listed in Table 2. The selected genotypes exhibited similar levels of sprouting resistance to durum check cultivar, Kyle. In 2001, DR-103 had the lowest sprouting score, while in 2002 several genotypes had the sprouting score of zero. Similar pattern was detected for sprouting index. Sprouting scores and sprouting indices of the accessions (not including the check) tested in 2001 and 2002 were correlated significantly ($r = 0.80$ and 0.88, $n = 28$). This indicates consistency in ranking across environments. The effects of the environment encountered by the maternal parent during seed development on the susceptibility to pre-harvest sprouting have been reported for a wide range of species (Fenner, 1991; Wulff, 1995). Among the different factors acting on the mother plant, temperature appears to be the primary determinate of year-to-year variation in pre-harvest sprouting (Cochrane, 1993). With respect to high correlation among screening methods in 2001 and 2002, environmental factors might not affect sprouting behavior of accessions. Selected genotypes indicate that incorporating resistance to pre-harvest sprouting into desirable wheat phenotypes, is an attainable objective. Lower pre-harvest sprouting scores, indices and α-amylase activity showed that genes for resistance exist in germplasm and could effectively transferred to other genotypes.

There are few reports in the literature concerning genetic variation in pre-harvest sprouting resistance of tetraploid wheat. Earlier attempts to increase the level of sprouting resistance of tetraploid wheat have been based on the transfer of resistance from red grained bread wheat. The use of tetraploid sources with high levels of resistance would be genetically easier (Clarke *et al.*, 1994). Moreover, the availability of genetic sources of resistance, combined with suitable selection schemes, should however, speed progress toward a solution. In this

Table 2: Twenty-eight durum genotypes with the lowest average sprouting score for 2001 and 2002 compared to several common and durum check cultivars for sprouting score, sprouting index and α-amylase activity

	Sprouting score‡		Sprouting index‡		α-amylase activity
	2001	2002	2001	2002	2002
Genotype†	Scale of 1 to 10		(%)		s
RL4137	2	0	6	0	360.33±5.21
AUS1293	0	3	0	6	380.00±5.77
AUS1408	1	0	2	0	393.00±1.76
Kyle	0	3	0	10	420.41±6.94
DR-10	5	0	10	1	465.33±7.84
DR-11	5	0	12	1	430.00±11.30
DR-32	5	0	10	0	463.33±1.53
DR-37	5	2	10	4	470.33±2.85
DR-40	5	1	10	2	605.00±2.08
DR-43	2	1	8	2	329.67±9.07
DR-44	4	2	8	4	497.33±12.30
DR-45	3	2	10	4	549.33±8.69
DR-68	4	2	14	4	359.67±5.77
DR-73	4	1	8	1	530.33±8.89
DR-89	4	0	8	1	459.67±10.60
DR-103	0	1	0	1	441.33±13.60
DR-160	5	0	10	1	485.33±16.10
DR-202	4	2	8	3	429.67±11.50
DR-218	3	1	6	1	617.67±6.49
DR-238	2	0	4	1	471.33±28.90
DR-248	4	2	8	5	478.00±15.10
DR-255	2	1	4	3	458.67±8.54
DR-262	5	2	10	4	385.33±13.00
DR-263	4	0	8	0	513.67±14.50
DR-268	5	1	10	1	372.67±8.50
DR-304	4	2	8	3	566.00±11.70
DR-308	3	0	6	0	438.67±7.31
DR-316	4	2	8	3	310.00±14.90
DR-317	3	1	10	2	556.67±21.10
DR-380	5	2	10	3	570.33±5.61
DR-387	3	2	6	3	339.33±10.80
DR-396	5	0	10	1	466.67±5.29

† Numbers are from the College of Agriculture, University of Tehran Germplasm Bank. RL4137 is red common wheat, AUS1293 and AUS1408 are white common wheats and Kyle is durum wheat. ‡ One replicate, one harvest time in 2001, mean of three replicates and one harvest time in 2002

study a wide range of sprouting resistance was detected. The durum genotypes ranged from zero to ten and zero to 100 for sprouting score and sprouting index, respectively. In a study by Mares (1987), 80 wheat accessions from eight tetraploid species were evaluated for pre-harvest sprouting resistance. Similar to the results of this study, two tetraploid species, *T. durum* and *T. polonicum* contained promising accessions for sprouting resistance. In another study, a collection of durum wheat germplasm from ICARDA and Spain was evaluated by Clarke *et al.* (1994) for sprouting resistance. In agreement with the present study, Stewart 63, considered to be a sprouting resistant genotype. In contrast, Kyle reported to be a genotype with an intermediate level of sprouting resistance, considered highly resistant in the present study.

Analysis of variance detected significant differences among genotypes for falling numbers. In this study high falling numbers were detected for all genotypes. A number of durum genotypes had higher falling number compared to durum and hexaploid wheat controls. DR-218 and DR-43 had the highest and the lowest falling numbers, respectively. Low falling number in wheat is an intermittent problem in humid areas. Pre-harvest prediction of falling number may be of value to the producer to make better-informed decisions on the cost and benefit of an early, high grain moisture harvest. There are four ways in which α-amylase might develop and lead to a low falling number (Kettelwell *et al*., 1998). Three of these are detectable well before harvest. Pre-maturity α-amylase activity is one indicator. In this study, spikes were harvested at ZGS92 and since temperature fluctuations during grain development can trigger the formation of pre-mature α-amylase, falling number assays were performed. The high falling numbers of the durum accessions (Table 2) indicate that there is no risk of pre-mature α-amylase activity. In spite of high falling numbers for durum accessions, sprouting score and sprouting index values in 2002 were not correlated with α-amylase activity ($r = -0.09$ and $r = -0.11$, $n = 28$). Similar to our results, Rajaram *et al.* (1992), observed no significant correlation between pre-harvest sprouting and α-amylase activity. In contrast to our results, DePauw and McCaig (1991) reported that sprouting score, percentage sprouted kernels and α-amylase activity were highly correlated. Clarke *et al.* (1994) found a moderate correlation between mean sprouting score and α-amylase activity. However the aforementioned reports did not indicate whether or not the measured enzyme activity is indicator of pre-mature α-amylase activity. According to Upadhyay and Paulsen (1988) α-amylase is probably not an appropriate selection criterion for pre-harvest sprouting resistance because of the relatively low correlation with visual sprouting after simulated rain.

There are two important questions that are answered. First what is the genetic variability of the resistance to pre-harvest sprouting and secondly how reliably can pre-harvest sprouting be predicted using various screening methods. Based on the results, it can be concluded that most of the durum lines in this study have a low to moderate level of pre-harvest sprouting resistance. The durum lines with high level of sprouting resistance selected in this study can provide breeding material for improvement of pre-harvest sprouting tolerance. Highly significant correlation among two screening methods, namely, sprouting score and sprouting index, suggesting that for evaluation a large number of accessions, sprouting score can be applied as an easy, fast and reliable method.

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