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Application of Projected Pollen Area Response to Drought Stress to Determine Osmoregulation Capability of Different Wheat (*Triticum aestivum* L.) cultivars

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ABSTRACT

Six different wheat cultivars were compared in terms of osmoregulation capability, using the ratio of the projected pollen area under normal and water stress conditions. Cultivars Asakaze, BR10 and sardary which had ratios less than one were classified as the low osmoregulation groups while BR9, Sabalan and Alvand which had ratios more than one were grouped as high ones. The results were confirmed as compared by the results of the changes of flag leaf water and osmotic potential during a period of progressing water stress. The technique seems to be promising for distinguishing the cultivars capability of osmoregulation.

Key Words: Pollen area; Drought stress; Osmoregulation; Wheat

INTRODUCTION

Maintenance of high turgor is very important for normal functioning of the plant cell under water stress condition. Osmotic adjustment is considered as one of the mechanisms that maintains high turgor in droughted plants (Morgan, 1980). As a result stomata remain opened and gas exchange takes place normally. Increasing in the cell solute content is defined to be the cause of the osmotic adjustment (Morgan, 1992). Large genotypic variation has been noted for osmoregulation in flag leaves, spikelets, and growing grains of wheat (Morgan, 1980). Selected lines for high osmoregulation derived from a cross between two contrasting parents showed the ability to maintain greater turgor compared to unselected control ones and produced 1.6 times more grain yield (Morgan, 1983).

Measurement of osmoregulation in F2 lines derived from a cross between a high and low osmoregulation lines showed two overlapping distributions indicating that a single recessive gene is responsible for high osmoregulation (Morgan, 1991). Results of substitution lines also showed that the gene is located on chromosome 7 in genome A (Morgan, 1980), which is 13-cM away from RFLP locus Xpsr119 towards the centromere (Morgan, 1996).

Direct measurement of osmoregulation is however difficult since the measurement should be done on many samples over a relatively long period of developing stress and needs the worker to be very skillful using the available technology. Sampling technique is also very important because osmotic potential is affected by the time, the tissue and even by the instrument, which is used for the sampling.

As a surrogate of direct measurement of osmoregulation, coleoptile and root length responses to the stress have been proposed to be measured in wheat (Morgan, 1988). Since almost 87% of the concentrating solutes in the cells is potassium, measurement of its concentration in the tissue sap is used instead of the direct measurement of osmoregulation (Morgan, 1992). RFLP locus Xpsr119 is can also be used as a marker to select for osmoregulation in wheat breeding programs (Morgan, 1996). Dough strength of grain flour is employed as an effective way to distinguish the capability of osmoregulation of the cultivars since the Per-A4 locus which is responsible for peroxidase activity and affects the dough strength is shown to be linked to the *or* locus (Morgan, 1999b). Recently measurement of projected pollen grain area method has been proposed as a technique to identify and select the recessive homozygous from the heterozygous in the backcross breeding programs (Morgan, 1999a). The technique is very easy though it needs plants to reach the anthesis. The aim of this study was to measure the response of different wheat cultivars in terms of projected area of pollen grains to the water stress and compare the results with leaf osmoregulation under increased stress conditions.

MATERIALS AND METHODS

Six wheat cultivars that represent a wide range of genetic variation were selected. Cultivar's characteristics and the methods have been described in a companion paper. Briefly the experiment was started in a glasshouse and continued in a growth cabinet. Seeds were sown in Wagner pots (1/2000) at 3-cm depth. Plants were transferred to a growth cabinet after they reached the stem elongation period and the water stress was applied by withholding the water.

Water status of the plants was measured at 24 h

intervals by measuring, leaf water potential (Ψ_w) and leaf osmotic potential (π). Each time a fully expanded leaf was selected and a 5mm diameter leaf disk was cutted and immediately inserted into a C-52 Wescor chamber to measure Ψ_w. Psychrometric operating method was used in both water and osmotic potential measurements and the chambers were calibrated separately using the standard NaCl solutions at room temperature according to the manual of Wescor. A 5cm long segment was also cut from the same leaf and immediately inserted in a vinyl bag, sealed and transferred to a deep freezer (-82ºC) for later measurement of π. After freezing and thawing of the samples π was measured by inserting a paper disk inside the vinyl bag and saturating it with the tissue sap by pressing the leaf with a pen. Turgor pressure was calculated as the difference between Ψ_w and π. All samples were taken from middle parts of the leaves 4 to 5 hours after starting the light period.

One set of pots was kept well watered until anthesis. Pollen grains of matured but not opened anthers were soaked into 30 and 50% polyethylene glycol (6000) (PEG) solutions over a microscope slide according to Morgan (Morgan, 1980) in order to check for the possible existence of the gene *or* which, in it's recessive homozygous form (*oror*) is responsible for osmoregulation in wheat. KCl was added to the solutions at 10 mmol level. Cultivars were considered to be homozygous for *or* locus. Slides were covered and after 24 h incubating in room temperature were examined by a microscope at ×200 magnification. Six photos were taken from the best-arranged pollen grains at the middle part of each slide. After printing, the photos were scanned and the projected area of each pollen grain was measured using ScionImage computer program while scale was set at micrometer. The ratio between the pollen projected area under 50% to 30% PEG solution was then calculated for each cultivar.

RESULTS AND DISCUSION

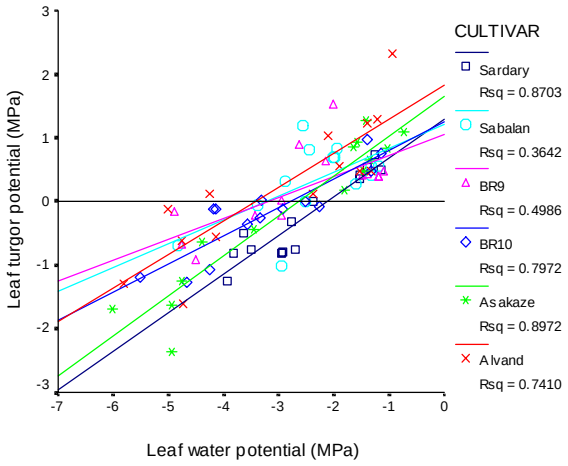
Pollen area ratio under 50 to 30% PEG solutions was less than 1 for cultivars Asakaze, BR10 and Sardary while it was higher than 1 for BR9, Sabalan and Alvand (Table I) showing that the later group is osmoregulation capable while the former is not. These results suggest that the first group be dominant homozygous for *or* locus and as a result capable for osmoregulation while the second group is recessive and not capable (Morgan, 1999b). Fig 1 shows that when leaf water potential decreases during stress period, leaf turgor potential also significantly decreases in all cultivars. Cultivars reached to zero torgur at different leaf water potential (Table I, Fig 1). Predicted values of leaf water potential at zero turgor and their 95% confidence intervals are also showing that the cultivars could be separated into almost the same distinct groups (Table I).

The promising method for distinguishing the cultivars capability of osmoregulation, thereby could be used to identify the cultivars, for use as the donor parent in backcross breeding programs. For instance cultivars BR9, Alvand and Sabalan could be used for this purpose.

Table I. Projected area (μm^2) and area ratios of pollen grains of wheat cultivars grown under non-stressed condition after being soaked in 50% and 30% PEG solutions

Cultivar	50%	30%	50/30
Alvand	2238.46	1966.88	1.14
Asakaze	2186.32	2238.89	0.98
BR10	1825.86	1959.83	0.93
BR9	2246.58	1892.86	1.19
Sabalan	1972.59	1825.66	1.08
Sardary	2177.99	2486.92	0.88

Fig. 1. Relationship between leaf water and torgur potential for wheat cultivars during a period of water stress. Black horizontal line represents the zero torgur potential.



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