9. Patrick, K., Calfas, G. J., Zabinski, M. F. and Cella, J., Diet, physical activity and sedentary behaviours as risk factors for overweight in adolescence. *Arch. Pediatr. Adolesc. Med.*, 2004, **158**, 385–390.
10. National Council for Applied and Economic Research, National Human Development Report, Oxford University Press, Oxford, UK, 2001.
11. Klesges, R. C., Shelton, M. L. and Klesges, L. M., Effects of television on metabolic rate: potential implications for childhood obesity. *Pediatrics*, 1993, **91**, 281–286.
12. Freedman, D. S., Dietz, W. H., Srinivasan, S. R. and Berenson, G., The relation of overweight to cardiovascular risk factors among children and adolescents. The Bogalusa Heart Study. *Pediatrics*, 1999, **103**, 1175–1182.
13. Chhatwal, J., Verma, M. and Riar, S. K., Obesity among pre-adolescent and adolescents of a developing country (India). *Asia Pac. J. Clin. Nutr.*, 2004, **13**, 231–235.
14. Ramachandran, A. *et al.*, Prevalence of overweight in urban Indian adolescent school children. *Diabetes Res. Clin. Pract.*, 2002, **57**, 185–190.
15. Marwaha, R. K., Tandon, N., Singh, Y., Aggarwal, R., Grewal, K. and Mani, K., A study of growth parameters and prevalence of overweight and obesity in school children from Delhi. *Indian Pediatr.*, 2006, **43**, 943–952.
16. Khadilkar, V. V. and Khadilkar, A. V., Prevalence of obesity in affluent school boys in Pune. *Indian Pediatr.*, 2004, **41**, 857–858.
17. Kapil, U., Singh, P., Pathak, P., Dwivedi, S. N. and Bhasin, S., Prevalence of obesity amongst affluent adolescent school children in Delhi. *Indian Pediatr.*, 2002, **39**, 449–452.
18. Ge, K., Body mass index of young Chinese adults. *Asia Pac. J. Clin. Nutr.*, 1997, **6**, 175–179.
19. Ko, G. T. *et al.*, Simple anthropometric indexes and cardiovascular risk factors in Chinese. *Int. J. Obes. Relat. Metab. Disord.*, 1997, **21**, 995–1001.
20. Yoshiike, N., Matsumura, Y., Zaman, M. M. and Yamaguchi, M., Descriptive epidemiology of body mass index in Japanese adults in a representative sample from the National Nutrition Survey 1990–1994. *Int. J. Obes. Relat. Metab. Disord.*, 1998, **22**, 684–687.
21. Aekplakorn, W. *et al.*, Prevalence and determinants of overweight and obesity in Thai adults: results of the Second National Health Examination Survey. *J. Med. Assoc. Thai.*, 2004, **87**, 685–693.
22. Griffiths, P. L. and Bentley, M. E., The nutrition transition is underway in India. *J. Nutr.*, 2001, **131**, 2692–2700.
23. Chu, N. F., Prevalence of obesity in Taiwan. *Obes. Rev.*, 2005, **6**, 271–274.
24. Wu, Y., Overweight and obesity in China. *BMJ*, 2006, **333**, 362–363.
25. World Health Organization, Diet, nutrition and prevention of chronic diseases, Geneva, 2003.
26. Bar-Or, O. *et al.*, Physical activity, genetic, and nutritional considerations in childhood weight management. *Med. Sci. Sports Exerc.*, 1998, **30**, 2–10.

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Osmotic adjustment in pollen grains: a measure of drought adaptation in sorghum?

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The immediate and most common response by the different organs of a plant to water stress is decrease in turgor. This may be partially or fully adjusted by accumulation of solutes. In the present study sorghum pollen grains were subjected to *in vitro* osmotic stress using polyethylene glycol (PEG). The change in size and shape of the pollen grains under osmotic stress was considered as a measure of osmotic adjustment (OA). The *in vitro* pollen response to osmotic stress with and without osmolyte and genotypic response to pollen OA vis-à-vis leaf OA was analysed in kharif and rabi sorghum genotypes. At 40% PEG (discriminative osmotic stress), the pollen grains of rabi genotypes retained their size, whereas the kharif genotypes showed shrinkage but responded to external supply of osmolyte. This indicates increased capacity of turgor adjustment in rabi genotypes compared to kharif genotypes. The increased capacity of turgor adjustment is referred to as intrinsic OA and the response to external supply of osmolyte as induced OA. The leaf OA was significantly high in rabi genotypes compared to kharif genotypes, indicating a close correspondence between intrinsic OA in pollen grains and high leaf OA. In addition the study indicates that OA is a drought-adaptive trait and could have evolved in the rabi genotypes by virtue of their regular exposure to moisture stress, and it could be induced in kharif genotypes.

Keywords: Drought adaptation, osmotic adjustment, pollen grain, sorghum.

MANY physiological mechanisms are adapted by crop plants to drought stress. Integration of these traits in breeding programmes is difficult due to complex or time-consuming protocols[1]. Osmotic adjustment (OA) is one of such mechanisms, which is routinely used to test the drought tolerance of the genotype[2]. It is normally estimated by Morgan's regression method[3], Ludlow's full turgor adjustment method[4] or rehydration method[5,6]. All the three methods require estimation of osmotic potential and relative water content with change in leaf water potential. As a result, this method is time-consuming and difficult for screening a large number of genotypes.

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Pollen grains of flowering plants function as simple, autonomous individuals for short duration. In a number of situations pollen testing has proven to be an effective alternative for sporophytic testing of resistance to biotic and abiotic stress factors[7–9]. The effect of stress on pollen genotype and pollen response was measured in terms of pollen viability, germination and tube growth[10–12]. However, Morgan[13] reported that changes in the *in vitro* pollen grain size and shape under osmotic stress can be used as a measure of OA in pollen grains, which in turn was related to sporophytic OA. Sorghum pollen grains are round in shape, numerous and the change in size and shape can be easily observed under a microscope. The pollen grains of different genotypes of sorghum were exposed to osmotic stress and the resultant changes in size and shape of pollen genotypes were measured under a microscope as an indication of pollen OA. In the present study, we specifically asked the questions: Does OA play a critical role in maintaining the size and shape of pollen grains under stress? And does it reflect the sporophytic OA?

In India sorghum is grown both in rainy (kharif) and post-rainy (rabi) seasons, predominantly under rainfed conditions. In rabi season the crop is grown under residual moisture condition and constantly experiences moisture stress at flowering and grain-filling stage (Figure 1). On the other hand, kharif-grown crops suffer from intermittent drought stress at different growth stages, and the time and duration of stress are not constant. The two distinct growing conditions led to selection of different genotypes for the two seasons. It is expected that the genotypes grown during the two seasons adapt different strategies for moisture stress tolerance.

Six sorghum genotypes, viz. E36-1, SPV 86, Sel. 3, GRS 1, AJ 2113 and M 35-1 grown and adapted to post-rainy (rabi) season and two genotypes, viz. DSV 1 and DSV 2, adapted to rainy season (kharif) were chosen for the study. The selected genotypes were grown with (control) and without irrigation (stress) during post-rainy season of the year 2004–2005 (date of sowing 14 September 2004) for measuring leaf OA. Each genotype was grown in two rows of 2.5 m length, spaced 30 cm apart, and replicated twice in both moisture conditions. The non-stress (control) condition consisted of irrigating the crop at regular interval of 8–10 days from sowing, till physiological maturity. The moisture stress treatment was imposed by withholding the irrigation 15 days after sowing.

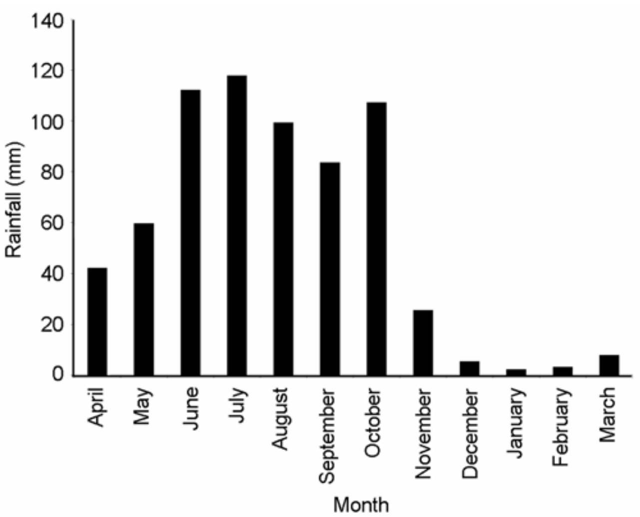

Figure 1. Month-wise average rainfall in sorghum-growing areas of South India (20 years average). June to November, kharif season and October to February, rabi season.

Leaf discs of 1 cm diameter were taken from the third fully opened leaf from the top. At 70 days after sowing five plants were chosen randomly in each genotype and in each replication of both the main treatments for taking the leaf disc. The sap from the frozen leaf discs was quickly extracted by centrifuging at 10,000 rpm. The osmotic potential of the sap was determined using Wescor 5520 vapour pressure osmometrer (Wescor, USA). The relative water content of each genotype in both treatments was recorded[14]. The OA of a genotype was calculated as the difference between osmotic potential (adjusted for relative water content) in non-stress and moisture stress treatment[4]. For each genotype and treatment, five samples were drawn. The replicated data were subjected to analysis of variance using complete randomized design and the genotypic means were subjected to Duncan's multiple range test at 5% probability to test the genotypic differences to leaf OA.

The critical concentration of osmoticum (polyethylene glycol; PEG) for induction of stress on pollen grains under *in vitro* conditions was determined as follows. All genotypes were grown in the field during rabi season of 2004 without moisture stress. The pollen grains were collected from the freshly dehisced anthers.

The sorghum pollen grains were exposed to a wide range of osmotic stress using different concentrations of PEG-6000 in cavity slides. The pollen grains were incubated in 50 μl of PEG solution for 24 h at 70–80% relative humidity under room temperature. The concentration of PEG ranged from 0% to 50% at an interval of 4%. The diameter of the pollen grains was measured immediately after dispensing in PEG medium and after 24 h on a projection microscope screen with a magnification of 400×. At lower concentration (< 30%) the pollen grains showed bursting. At increased concentration of PEG, the pollen grains showed uniform shrinkage (data not shown). Forty and fifty per cent PEG were considered as critical concentrations for induction of osmotic stress in pollen grains.

Two concentrations of PEG were selected for studying the response of pollen genotypes to osmotic stress. The pollen size was measured using compound microscope with a projection screen at a magnification of 400×. The outlines of 25 pollen grains from four cavities were traced on the projection screen and the areas of the tracings were recorded using UVI image analysis system (UVI.DOC DOC-008-XD). The area of the pollen grains was measured immediately after dispersing onto the PEG for normal size of the pollen grains and after 24 h incubation at 70–80% RH under room temperature for a size under osmotic stress. The area of the pollen grains was expressed in terms of number of pixels covered by each pollen grain. The ratio of pollen size under osmotic stress to its normal size was taken as a measure of intrinsic OA in the pollen grains.

We have studied the response of pollen grains to osmolyte $CaCl_2$ at various concentrations (data not shown). $CaCl_2$ at a concentration of 10 mM was selected for the study. Pollen grains of all the genotypes were incubated in 40% and 50% PEG containing 10 mM $CaCl_2$. Five cavities were prepared for each genotype and five pollen grains from each cavity were randomly selected for recording observations immediately after dispersing the pollen grains on the medium and 24 h after incubation.

The following pollen parameters were determined for all the genotypes.

A – Normal size of the pollen grains: The pollen grains were dispersed onto the cavity slides containing 40% PEG solution and immediately the size of the pollen grains was measured as mentioned earlier.

B – Effect of osmotic stress on pollen grains: The size of the pollen grains in 40% and 50% PEG after 24 h of incubation.

C – Response to osmolyte: The size of the pollen grains in 40% and 50% PEG with 10 mM $CaCl_2$ solution after 24 h of incubation.

The ratio of pollen parameters (*A*, *B* and *C*) was used to determine the mechanism of OA in the pollen grains of different genotypes as given below:

$B/A \cong 1$ – Intrinsic OA; $B/A < 1$ – No intrinsic OA; $C/A \cong 1$ – Uptake of osmolyte for OA and $C/A < 1$ – No uptake, no OA.

The replicated data of pollen size ratio at 40% and 50% PEG, with and without $CaCl_2$, were subjected to Duncan's multiple range test to examine the difference between kharif and rabi genotypic means.

Crop plants have adapted different mechanisms to cope with drought patterns. OA is receiving increasing recognition as a major strategy for drought tolerance. Genetic variation for OA has been reported in a number of crops[15,16]. OA operates mainly under stressful conditions and therefore yield potential is not affected[17]. Also, it is common among all crop plants[18], including cellular organisms. Consequently, OA was associated with increased yield under stress in peas[19], chickpea[20], brassica[21], wheat[17] and sorghum[22,23]. In the present study the leaf OA was estimated for six rabi and two kharif season-adapted genotypes. We measured the leaf OA by conducting a field experiment under stress and non-stress environments. The mean leaf OA of rabi season genotypes was significantly higher than kharif season genotypes (Table 1). The analysis of individual genotypes suggested that all the rabi genotypes had significantly higher OA values compared to kharif genotypes, except selection 3. The within-group variation was not significant. The results categorically differentiated the kharif and rabi season-adapted genotypes for leaf OA (Table 1). Brown *et al.*[24] and Cutler and Rains[25] have shown that shoots have an increased capacity for turgor adjustment as the water potentials decline if they have been previously subjected to drought. This has also been observed in the leaves of cotton[24] and in the phyllodes of xerophyte, *Acacia harpophylla*[26]. Ackerson[27] has shown that cotton plants which were osmotically 'adapted' to a previous cycle of water stress, showed a lower threshold water potential for stomatal closure under subsequent water stress. Finger-millet seeds obtained from plants subjected to moisture stress showed higher germinability and seedling vigour under simulated stress. This was attributed to a lower solute potential of seeds resulting from the accumulation of solutes like sucrose[28].

Terminal drought is more in rabi season in all the years; consequently, the genotypes adapted to rabi season survive through terminal stress conditions (Figure 1). The kharif season genotypes fail to perform under rabi situations and are not suitable for growing during rabi season. OA may be one of the adaptive mechanisms evolved in rabi genotypes to combat the drought situation. During kharif season the drought is intermittent, and the time and duration of occurrence are uncertain. It is presumed that the kharif season genotypes adapted a different mechanism to combat unexpected spells of drought at any stage of growth.

Forty and fifty per cent PEG were used to test the response of pollen grains of selected genotypes to osmotic stress. Except GRS 1, all other rabi season genotypes retained near-normal size at 40% PEG (Table 2). The kharif season genotypes recorded a size ratio of 0.759,

Table 1. Leaf osmotic adjustment (OA) in sorghum genotypes

Genotype	Leaf OA (MPa)
Rabi genotype	
E36-1	0.243[a]
SPV 86	0.248[a]
Sel. 3	0.168[ab]
GRS 1	0.295[a]
RS 29	0.300[a]
M 35-1	0.286[a]
Mean	0.257
Kharif genotype	
DSV 1	0.088[b]
DSV 2	0.089[b]
Mean	0.088

Values with the same superscript do not differ significantly. The replicated data were analysed using CRD design to compare the genotypes.

Table 2. Pollen grain size of different genotypes under increased polyethylene glycol (PEG) stress

Genotype	Normal size (*A*)	Forty per cent PEG (B_1)	Fifty per cent PEG (B_2)	Size ratio B_1/A	Size ratio B_2/A
Rabi					
E 36-1	11560.00	10492.67	8746.00	0.908	0.757
SPV 86	10928.00	9206.75	7727.75	0.842	0.707
Sel. 3	11020.75	12081.33	8174.00	1.096	0.742
GRS 1	11709.00	8960.00	7750.67	0.765	0.662
AJ 2113	11323.20	12267.40	8999.75	1.083	0.795
M 35-1	11022.67	9711.00	8605.80	0.881	0.781
Mean				0.929^b	0.741^a
Kharif					
DSV 1	9163.00	6866.33	6446.00	0.749	0.703
DSV 2	9532.50	7322.50	6809.00	0.768	0.714
Mean				0.759^a	0.708^a

Size of the pollen grains was measured as area covered by the number of pixels per unit square in gel documentation. Values with different superscripts differ significantly.

indicating the reduction in pollen size. With the increase in concentration (50% PEG) reduction in pollen size was observed for both rabi (0.741) and kharif (0.708) season genotypes. The results indicate that the pollen grains of rabi season genotypes maintain their size at 40% PEG, suggesting tolerance to osmotic stress. On the other hand, the pollen grains of kharif season-adapted genotypes were susceptible to stress and their size was significantly reduced when exposed to moisture stress. The retention of pollen grain size in rabi genotypes under increased PEG stress could be primarily due to higher concentration of solutes accumulated by their regular cultivation under residual moisture condition and exposure to post-flowering moisture stress as in the case of cotton[25,27] and finger millet[28]. Such adjustment prevents the loss of turgor and maintains the pollen/cell size, and physiological activity will be maintained at low water potential.

Various mechanisms operate for OA in plants – synthesis of organic solutes and/or accumulation of cations like Ca^+ and K^+ in the cytoplasm, either through release of membrane-bound cations or translocation across the plasma membrane[29]. The efficiency of pollen grains to accumulate cations through transmembrane movement from the external medium was evaluated by supplementing the PEG medium with osmolyte.

The response of pollen grains to osmolyte ($CaCl_2$) under two concentrations of PEG (40% and 50%) was studied. At both 40% and 50% PEG, the kharif season genotypes increased their size when supplemented with $CaCl_2$ (Table 3). The size ratio of the kharif genotypes was approximately equal to one at 40% PEG. Addition of osmolyte had no influence on the maintenance of size and shape in rabi genotypes. Surprisingly, the pollen grain size of rabi genotypes at 40% PEG supplemented with osmolyte was less. On the other hand, the rabi season-adapted genotypes maintained their size at 40% PEG stress in the absence of osmolyte. The results suggest that the mechanism of OA is different in kharif and rabi genotypes, at least in pollen grains.

Critical analysis of all the genotypes revealed that the retention of pollen grain size in rabi genotypes at 40% PEG could be because of biochemical changes and/or pre-synthesized biochemicals leading to intrinsic OA. Such a mechanism could have been evolved in rabi-adapted genotypes by virtue of their exposure to recurring receding moisture conditions. The reduction in pollen grain size of kharif genotypes in the absence of an external supply of a osmolyte indicates the absence of intrinsic adaptive mechanism. However, in the presence of external osmolyte (cation), they retain their size, indicating uptake of cations through the plasma membrane. It appears from the results that the rabi sorghum adapted a more consistent mechanism of OA, whereas the adaptation in case of kharif genotypes is uncertain and depends on external factors. The pollen response of kharif genotypes resembles osmoregulation in the bacterium, *Escherichia coli* and wheat which involves a high-affinity potassium uptake response to osmotically induced water stress[13,30].

The comparison of pollen response to osmotic stress and leaf OA reveals that the rabi genotypes have high leaf OA and their pollen grains retained their size and shape under discriminative osmotic stress of 40% PEG (Table 4). The kharif genotypes recorded low leaf OA and shrinkage of pollen grains at 40% PEG. Sorghum genotypes grown under rabi season invariably experience moisture deficit at the flowering stage, leading to adverse effect on grain yield. The immediate and most common response by the different organs of the plant to moisture stress is decrease in turgor. This may be partially or fully adjusted by the accumulation of solutes, resulting in the maintenance of turgor even at low water potential. Examples for this OA have been presented for leaves[31], expanding hypocotyls[32], roots[33], the reproductive apex[34], spikelets[35]

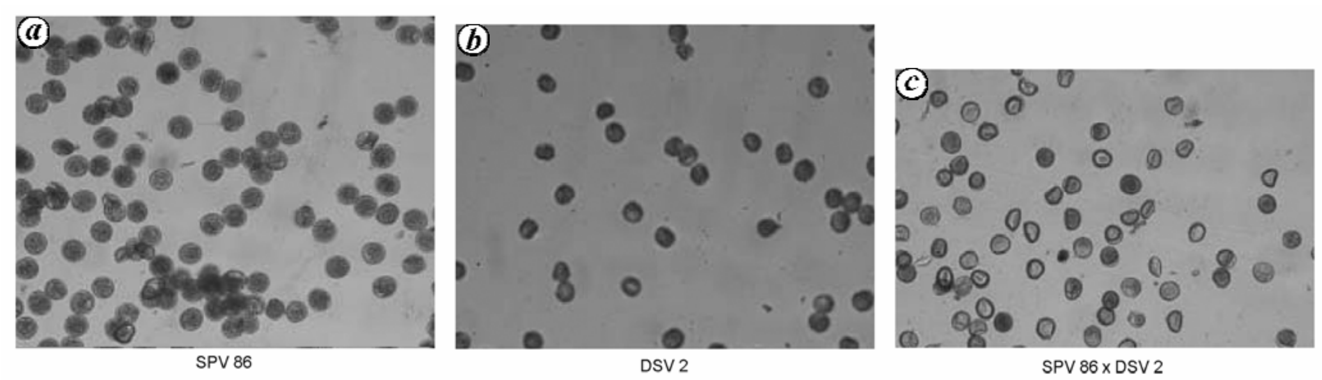

Figure 2. Response of pollen grains to 40% PEG stress. ***a***, Maintenance of pollen grain size and shape of rabi genotype SPV 86. ***b***, Shrinkage of pollen grains of kharif genotype DSV 2. ***c***, F_2 pollen grains produced by F_1 (SPV 86 × DSV 2) showing segregation for pollen size and shape.

Table 3. Pollen grain size of different genotypes under increased PEG stress supplemented with $CaCl_2$

				Size ratio	
Genotype	Normal size (A)	Forty per cent PEG (C_1)	Fifty per cent PEG (C_2)	C_1/A	C_2/A
Rabi					
E 36-1	11560.00	9208.80	9404.00	0.796	0.814
SPV 86	10928.00	8808.60	7705.67	0.806	0.705
Sel. 3	11020.75	8882.72	8137.67	0.806	0.738
GRS 1	11709.00	7507.50	7978.50	0.641	0.681
AJ 2113	11323.20	8224.25	7803.67	0.826	0.689
M 35-1	11022.67	9311.00	9348.34	0.845	0.833
Mean				0.771^{a}	0.743^{a}
Kharif					
DSV 1	9163.00	9276.50	8741.67	1.012	0.954
DSV 2	9532.50	8824.20	8024.50	0.926	0.842
Mean				0.969^{b}	0.898^{a}

Size of the pollen grains was measured as area covered by the number of pixels per unit square in gel documentation. Values with different superscripts differ significantly.

Table 4. Comparison of pollen and leaf response to osmotic stress

Genotype	Intrinsic OA	Leaf OA	Response to osmolyte
Rabi			
E 36-1	Present	High	No response
SPV 86	Present	High	No response
Sel. 3	Present	High	No response
M 35-1	Present	High	No response
Kharif			
DSV1	Absent	Low	Response
DSV2	Absent	Low	Response

and pollen grains[13]. The genotypes with high leaf OA produced pollen grains with tolerance to high osmotic stress. On the contrary, the kharif genotypes produced susceptible pollen grains, a reflection of poor intrinsic OA in their sporophyte.

The objective of OA is to retain the cellular functions under osmotic stress condition, measured indirectly in the form of maintenance of pollen grain size and shape under stress. The results indicate that it is possible to measure the OA as an indicator of moisture stress tolerance in sorghum genotypes through *in vitro* pollen bioassay and reinforce our view of using pollen selection as a tool in breeding for stress tolerance in crop plants[36,37]. Morgan[13] showed a single gene (*or*) conditioning differences in osmoregulation in wheat leaves which is also expressed in pollen grains. The technique is simple, fast and a large number of genotypes can be tested in less time and space. However, it is not clear from the present study whether the pollen OA depends on the pollen genotypes and/or the genotype of the sporophyte. However, a pilot study conducted suggested that OA in pollen grains was influenced by the pollen genotype instead of the sporophyte producing pollen grain. The hybrid plants were produced by crossing rabi (SPV 86) × kharif (DSV2)-adapted genotypes. The hybrid plants produced pollen grains which showed segregation for tolerance to moisture stress (Figure 2). When the pollen grains produced from F_1 were exposed to 40% PEG, they showed segregation for reduction in size and shape. Is this an indication of pollen genotype? The study is in progress.

1. Khan, H. R., Paull, J. G., Siddique, K. H. M. and Stoddard, F. L., Faba bean breeding for drought-affected environments: a physiological and agronomic perspective. *Field Crop Res.*, 2010, **115**, 279–286.
2. Seki, M., Umezawa, T., Urano, K. and Shinozaki, K., Regulatory metabolic networks in drought stress responses. *Curr. Opin. Plant Biol*., 2007, **10**, 296–302.
3. Morgan, J. M., Osmotic components and properties associated with genotypic differences in osmoregulation in wheat. *Aust. J. Plant Physiol*., 1992, **19**, 67–76.
4. Ludlow, M. M., Chu, A. C. P., Clements, R. J. and Kerslake, R. G., Adaptation of species of *Centrosema* to water stress. *Aust. J. Plant Physiol*., 1983, **10**, 119–130.
5. Blum, A., Osmotic adjustment and growth of barley genotypes under drought stress. *Crop Sci*., 1989, **29**, 230–233.
6. Blum, A. and Sullivan, C. Y., The comparative drought resistance of land races of sorghum and millet from dry and humid regions. *Ann. Bot.*, 1986, **57**, 835–846.
7. Ravikumar, R. L. and Patil, B. S., Pollen response as marker (PRM) and pollen selection: novel tools in crop improvement. In *Plant Physiology; Characteristics, Breeding, and Genetics* (eds Dris, R. and Barry-Ryan, C.), Science Publishers, 2002.
8. Hormaza, H. and Herrero, M., Male gametophytic selection as a plant breeding tool. *Sci Hortic*., 1996, **65**, 321–333.
9. Karapanos, I. C., Akoumianakis, K. A., Olympios, C. M. and Passam, H. C., Tomato pollen germination in relation to *in vitro* germination and pollen tube growth under favourable and stress-inducing temperatures. *Sex Plant Reprod.*, published online 10 January 2010.
10. Tuna, A. L., Burun, B., Yokas I. and Coban, E., The effects of heavy metals on pollen germination and pollen tube length in the tobacco plant. *Turk. J. Biol*., 2002, **26**, 109–113.
11. Ravikumar, R. L. and Chikkodi, S. B., Association between sporophytic reaction to *Alternaria helianthi* and gametophytic tolerance to pathogen culture filtrate in sunflower (*Helianthus annuus* L.). *Euphytica*, 1998, **103**, 173–180.
12. Bino, R. J., Franken, J., Witsenboer, H. M. A., Hille, J. and Dons, J. J. M., Effects of *Alternaria alternate* f. sp. *Lycopersici* toxins on pollen. *Theor. Appl. Genet.*, 1988, **76**, 204–208.
13. Morgan, J. M., Pollen grain expression of gene controlling differences in osmoregulation in wheat leaves: a simple breeding method. *Aust. J. Agric. Res*., 1999, **50**, 53–62.
14. Barrs, H. and Weatherly, P. E., A re-examination of relative turgidity for estimating water deficits in leaves. *Aust. J. Biol. Sci*., 1962, **15**, 413–428.
15. Morgan, J. M., Osmoregulation and water stress in higher plants. *Annu. Rev. Plant Physiol*., 1984, **35**, 299–319.
16. Rhodes, D. and Samaras, Y., Genetic control of osmoregulation in plants. In *Cellular and Molecular Physiology of Cell Volume Regulation* (ed. Strange, K.), CRC Press, Boca Raton, 1994, pp. 347–367.
17. Morgan, J. M., Growth and yield of wheat lines with differing osmoregulative capacity at high soil water deficit in seasons of varying evaporative demand. *Field Crop Res*., 1995, **40**, 143–152.
18. Zhu, J. K., Hasegawa, P. M. and Bessan, R. A., Molecular aspects of osmotic stress in plants. *Crit. Rev. Plant Sci*., 1997, **16**, 253–277.
19. Rodriguez-Maribona, B., Tenorio, J. L., Conde, J. R. and Ayerbe, L., Correlation between yield and osmotic adjustment of peas (*Pisum sativum* L.) under drought stress. *Field Crop Res*., 1992, **29**, 15–22.
20. Morgan, J. M., A gene controlling differences in osmoregulation in wheat. *Aust. J. Plant Physiol*., 1991, **18**, 249–257.
21. Kumar, A., Singh, P., Singh, D. P., Singh, H. and Sharma, H. C., Differences in osmoregulation in *Brassica* species. *Ann. Bot.*, 1984, **54**, 537–541.
22. Santamaria, J. M., Ludlow, M. M. and Fukai, S., Contribution of osmotic adjustment to grain yield in *Sorghum bicolor* (L.) Moench under water limited conditions. I. Water stress before anthesis. *Aust. J. Agric. Res*., 1990, **41**, 51–65.
23. Ludlow, M. M., Santamaria, J. M. and Fukai, S., Contribution of osmotic adjustment to grain yield in *Sorghum bicolor* (L.) Moench under water limited conditions. II. Water stress after anthesis. *Aust. J. Agric. Res*., 1990, **41**, 67–78.
24. Brown, K. W., Jordan, W. R. and Thomas, J. G., Water stress induced alterations of the stomatal response. *Physiol. Plant.*, 1976, **37**, 1–5.
25. Cutler, J. M. and Rains, D. W., Effects of water stress and hardening on the internal water relations and osmotic constituents of cotton leaves. *Physiol. Plant.*, 1978, **42**, 261–268.
26. Tunstall, B. R. and Connor, D. J., Internal water balance of brigalow (*Acacia harpophylla* F. Muell) under natural conditions. *Aust. J. Plant Physiol*., 1975, **2**, 489–499.
27. Ackerson, R. C., Stomatal response of cotton to water stress and abscisic acid as affected by water stress history. *Plant Physiol*., 1980, **65**, 455–459.
28. Dinesh Kumar, S. P., Sashidhar, V. R., Prasad, T. G., Udaya Kumar, M. and Seetharam, A., Solute accumulation, solute potential, germinability and seedling vigour of seeds of finger millet (*Eleusine coracana* Gaertn.) raised under rain-fed conditions and under irrigation. *Plant Cell Environ*., 1987, **10**, 661–665.
29. Pandey, G., Reddy, M. K., Sopory, S. K. and Singla-Pareek, S. I., Calcium homeostasis in plants: role of calcium binding proteins in abiotic stress tolerance. *Indian J. Biotechnol*., 2002, **1**, 135–157.
30. Laimins, L. A., Rhodes D. B. and Epstein, W., Osmotic control of *kdp* operon expressed in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA*, 1981, **81**, 4746–4750.
31. Munns, R. and Weir, R., Contribution of sugars to osmotic adjustment in elongating and expanding zones of wheat leaves during moderate water deficits at two light levels. *Aust. J. Plant Physiol*., 1981, **8**, 93–105.
32. Meyer, R. F. and Boyer, J. S., Osmoregulation, solute distribution and growth in soybean seedling having low water potentials. *Planta*, 1981, **151**, 482–489.
33. Sharp, R. E. and Davies, W. J., Solute regulation and growth by roots and shoots of water-stressed maize plants. *Planta*, 1980, **147**, 43–49.
34. Munns, R., Brady C. J. and Barlow, E. W. R., Solute accumulation in the apex and leaves of wheat during water stress. *Aust. J. Plant Physiol*., 1979, **6**, 379–389.
35. Morgan, J. M., Osmotic adjustment in the spikelets and leaves of wheat. *J. Exp. Bot*., 1980, **31**, 655–665.
36. Ravikumar, R. L., Patil, B. S. and Salimath, P. M., Drought tolerance in sorghum by pollen selection using osmotic stress. *Euphytica*, 2003, **133**, 371–376.
37. Ravikumar, R. L., Patil, B. S., Soregaon, C. D. and Hegde, S. G., Genetic evidence for gametophytic selection of wilt resistant alleles in chickpea. *Theor. Appl. Genet*., 2006, **114**(4), 619–625.

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