Research Article

Pollen Viability an Index of Abiotic Stresses Tolerance and Methods for the Improved Pollen Viability

Muhammad Khuram Razzaq[1,2*], Saeed Rauf[1], Mohsin Khurshid[3], Shahid Iqbal[1,4], Javaid Akhter Bhat[2], Ayaz Farzand[5], Adeel Riaz[1,6], Guangnan Xing[2] and Junyi Gai[2]

[1]Department of Plant Breeding and Genetics, University College of Agriculture, University of Sargodha, Pakistan; [2]Soybean Research Institute, National Centre for Soybean Improvement, Nanjing Agricultural University, China; [3]College of Allied Health Professionals, Directorate of Medical Sciences, Government College University, Faisalabad, Pakistan; [4]Laboratory of Fruit Tree Biotechnology, College of Horticulture, Nanjing Agricultural University, Nanjing, China; [5]Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan; [6]Biotechnology Research Institute, Chinese Academy of Agricultural Sciences Beijing, 100081 China.

Abstract | Pollen represents the substantial stage in plants and fertile pollen are important for proficient plant reproduction. Abiotic stresses reduce the photosynthates production, thus genotypes also reduce the reserve mobilization for tapetum cells which induce the significant reduction in pollen fertility. Therefore, pollen fertility index can be exploited to discriminate resistant and susceptible genotypes under abiotic stresses. High heritability in the segregating generation warrants good selection response. Moreover, magnitude and type of genetic variation associated with pollen viability also important in improving the trait and breeding procedures to be adapted for the improvement of trait *per se*. Genetic studies results under heat stress in sunflower showed additive to partial dominance towards higher pollen viability in the inheritance showing that trait can be improved through selection in segregating generations. In this review, we have mainly considered the effect of abiotic stresses on pollen viability, selection and methods for the improved pollen viability and the management of heat stress.

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***Correspondence** | Muhammad Khuram Razzaq, Soybean Research Institute, National Centre for Soybean Improvement, Nanjing Agricultural University, China; **Email:** khuram.uos@gmail.com

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Introduction

High yielding crops with improved stability are requisite for food security which makes the farming systems less susceptible to climate change (Hussain et al., 2017). Pollen grain are the carrier of male gamete in plants and differ in various morphologies. The main function of the pollen is to transfer the male genetic material into embryo sac through a process called as "double fertilization" (Mendieta and Granados, 2015). Pollen viability effect by numerous factors, especially abiotic and biotic stresses. Interactions of the plant with environmental factors that cause negative effects on plant development and survival are abiotic stresses. These comprised of all nonliving factors that affect the normal array of plants adapted environment (Abiko et al., 2005). Current advancements in biotechnology and functional genomics drastically changed our competences for gene discoveries (Cramer et al., 2011). High- throughput omics and bioinformatics approaches are facilitating tools for the discovery of genes and their predicted function. Moreover, genome scale networks reconstructions are vital to quantify the relationship between phenotype and genotype (Feist et al., 2008). The plant reaction to stresses depends upon organ affected by stress, for instance; transcriptional responses are tissue specific (Dinneny, 2008). In addition, the duration of the stress has substantial effect on intricacy of response (Tattersall et al., 2007; Pinheiro et al., 2010).

The function of ABC genes was determined with their downstream genes in floral deterioration and the development of unisexual flower in *Arabidopsis*. Furthermore, the role of hormones, such as; gibberellic acid, auxin, jasmonic acid, brassinosteroids and ethylene in the formation of floral organs and fertility was assessed. Results explored that modification in gene regulation affected the biosynthesis of hormones, in their transport and consequently caused abnormal floral organ development, poor pollen formation, loss of stamens and carpels that leads to male/ female sterility (Smith and Zhao, 2016; Yan et al., 2012). Sterility made by abiotic stresses is generally in male flower formation; especially during meiosis, flower bud initiation, tapetum development and fertilization. Moreover, number of genes are involved in the hormonal signalling, cold tolerance, heat shock, metabolism of sucrose and tapetum developments are indispensable for the plants to sustain fertility under abiotic stresses (Semenov and stratonovitch, 2013). The environmental factors such as soil fertility, moisture and growth conditions including temperature, relative humidity affect the ability of parent plant to produce pollen and also affect both the size and numbers of seed produced. Pollen is specifically susceptible to low relative humidity; pollen hydration is on the basis of its water contents. Pollen has high water contents (ca. 60% by weight) and respond sensitively to environmental conditions that can be to endorse discount water contents drops ca. 30% by weight (Firon et al., 2012).

In short, the environmental conditions of the parent plant affect the number and quality of offspring. It has been known that environmental factors during pollen formation are ample to cause major differences in pollen performance (Delph et al., 1997). These factors reduce the pollen viability and could be exploited to discriminate crop genotypes against various infield stresses. Therefore, this review will also be emphasized; selection and methods for the improved pollen viability and management of especially heat stress because reproduction phase development in crop plants utmost susceptible to abiotic stresses and predominantly increased temperature.

Effects of environmental factors on pollen viability

Supra-optimal temperature and cold stress: The seed yield of crop species has been shown to affect by the supra-optimal temperature. Supra-optimal temperature decreased the pollen viability, retention of pollen in the anthers and pollen germination, when the temperature was greater than or equal to 32 °C ≥ in various crop species (Razzaq et al., 2017). The pollen abortion was noted at uninucleate stage due to abnormal development of tapetum cells, when the temperature was greater than 36 °C which caused yield failure in C3 specie *Brachypodium distachyon* (Harsant et al., 2013). Heat stress on pollen viability relates to modification in the carbohydrate metabolism of anther during its development. In favourable temperature conditions (28/22 °C), the starch accumulation in the pollen grains of tomato plant reaches maximum value 3 d before anthesis. Continuous contact of the tomato plant to high temperature (32/26 °C) reduced the starch concentration and also decreased the soluble sugars in the pollen grains and anther walls (Pressman et al., 2002). Impact of high temperature has been well demonstrated in various crop species. In wheat high temperature during floret development can cause complete sterility. For instance exposure of the wheat plant to heat stress (32 °C) for continuous three days during an early reproductive phase when pollen mother cells were dividing caused pollen sterility which resulted in significant decrease in seed setting. In tomato, a temperature of 32/ 26°C, 15 days prior to anthesis corresponding to the meiotic division in the anther have a significant impact over the pollen viability (Sato et al., 2002). Sorghum pollen killed at the temperature of ≥ 42 °C and night temperature 10 °C, during meiosis cause failure of pollen mother cells, without affecting female viability. In cowpea, healthy pollen has large quantities of proline and just before anthesis, proline constitutes 55% amino acids in the anthers and pollen grains. Proline played role in protecting pollen from heat-induced damage in germination. In the grain legumes, the production of pollen grains and pollination are extremely sensitive to numerous stresses; such as high temperature, chilling and drought (Nguyen and Blum, 2004). Achene yield in sunflower (*Helianthus annuus* L.) is polygenic trait; that effected by environmental factors (Razzaq et al., 2017; Riaz et al., 2019). In sunflower pollen fertility under abiotic stresses has been exploited as a criterion for discriminating the genotypes against heat stresses (Kalyar et al., 2013; Kalyar et al., 2014). In order to determine the sunflower pollen viability, parental plants were exposed to heat stress (47°C day/ 32°C night) just before the start of reproductive phase (45 days after sowing). At the time of anthesis, another was collected early in the morning; anthers of each genotype were put in petri dishes to dehiscence. The obtained pollen was stained with the tetrazolium chloride (TTZ) and various populations were evaluated by noting the %age of stained or unstained pollen (Figure1 and 2). It was also known that pollen fertility was high in genotypes having downward sunflower head position at the time of anthesis. Downward sunflower head position protected the pollen from high solar radiation as compared to the upward head position (Kalyar et al., 2014). The impact of heat stress on pollen viability and relevant traits in various crop species is summarized in Table 1.

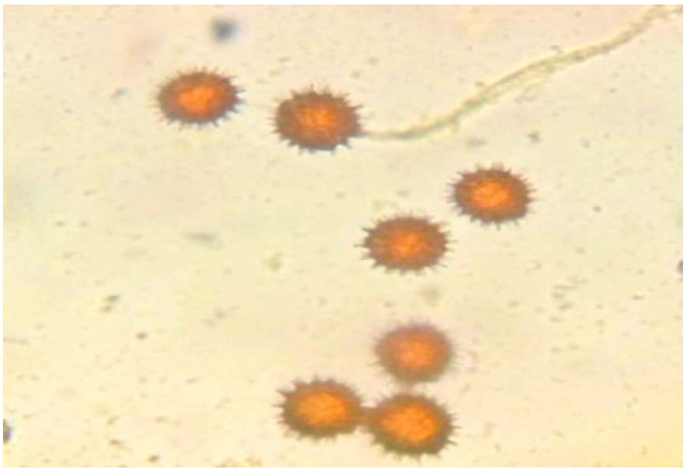

Figure 1: *High resolution (40x) staining of the sunflower (Helianthus annuus L.) pollen;* **Courtesy:** *Principal author.*

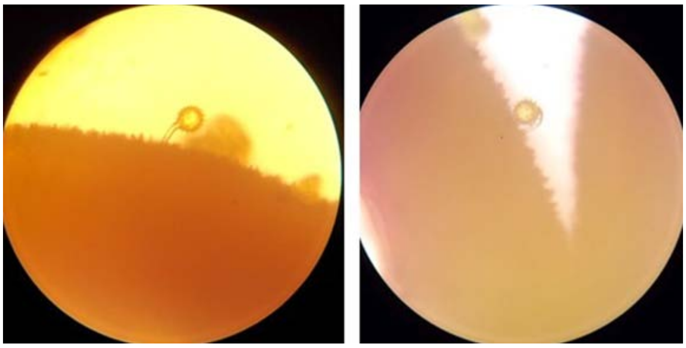

Figure 2: *Invivo heat resistant pollen germination and tube growth of sunflower (Helianthus annuus) through stigma.* **Courtesy:** *Principal author.*

During reproductive development, rice is more sensitive to low temperature. During microsporogenesis in rice, low temperature produces anther indehiscence and immature pollen, and it also reduces the anther length and number of pollen per anther, leading to male sterility. Rice varieties that have a great number of viable pollen shedding were more tolerant to cold stress (Satake, 1986). Another length, at low temperature, was related to a number of fertile pollen and low-temperature tolerance. Varieties that have long anthers are more tolerant to low temperature and this could be selection criteria in breeding. Male sterility by low temperature is the cause of reduced fertility but have no effect on the receptivity of stigma. A comparison was made between cold susceptible and resistant genotypes of chickpea (*Cicer arietinum*) under the low temperature of 15°C. Poor pollen germination due to restricted pollen tube growth was noted in cold susceptible varieties which resulted in fertilisation failures. On the other hand, cold resistant types had satisfactory pollen tube growth to cause effective fertilisation. It has been noted that pollen possessing the expression of WRKY34 alleles showed higher cold tolerance in Arabidopsis Zou et al. (2010). Integrating transcriptomic and proteomic study to demonstrate the mechanism of cold treatment in loquat, the results indicated the 1210 differentially expressed genes and 300 differentially expressed proteins. KEGG pathway showed the metabolic pathways and secondary metabolites were the greatest common pathways, in these transcriptional and translational processes (Lou et al., 2018).

Drought stress

Drought is the greatest limiting factor for agricultural production and the development of drought tolerant crop varieties is the economical approach to manage with drought (Qamar et al., 2018). Reproductive phase drought stress cause drying of anthers and decreases in pollen shedding, inhibit panicle exertion and increase sterility. Diminution in pollen viability or pollen activity has been indicated in a number of species specifically rice (Table 2). Pollen activity was shown through pollen ability to germinate or pollen load over the stigma. The studies have identified that seed yield was a function of pollen viability under stress condition (Nguyen et al., 2009; Fu et al., 2011; Gusmao et al., 2012; Sakhi et al., 2014). There was a close association between the genotypes for seed setting ability and pollen viability showing that route for higher yield under drought stress condition was due to higher gametophyte tolerance (Saragih et al., 2013; Sakhi et al., 2014).

Table 1: *Impact of heat stress on pollen viability and relevant traits in various crop species.*

Species	Provision of heat stress	Impact	Reference
Tomato (*Lycopersicon esculentum* L.)	15 days prior to anthesis	Significant reduction in the pollen viability	(Sato et al., 2002)
Groundnut (*Arachis hypogea* L.)	Dehiscenced anthers were exposed to the range of temperature from 10 °C to 47 °C with 2.5 °C interval	Genotypes were discriminated on the basis of pollen viability and pollen tube growth	(Kakani et al.,2002)
Sorghum (*Sorghum bicolor* L.)	38/21 °C day/night	Pollen viability and seed setting were significantly reduced. Pollen viability ranged (17-63%)	(Nguyen et al., 2012)
Soybean (*Glycine max* L.)	HT (38/28 °C) or optimum temperature (OT, 28/18°C) for 14 days at the flowering stage	Invitro pollen germination reduced by 22.7%. Deformation with thicker exine and disintegrated tapetum	(Djanaguiraman et al., 2013)
Sunflower (*Helianthus annuus* L.)	45°/ 30°C during the reproductive phase	Pollen sterility was used to discriminate heat tolerant and susceptible genotypes	(Kalyar et al., 2013)
Rice (*Oryza sativa* L.)	Six different day/ night temperature regimes (25/15, 30/20, 35/25, 40/30, 45/35 and 50/ 40 °C with a constant diurnal temperature variation of 10°C) for three days during panicle initiation	The decrease in pollen viability, pollen per anther, tube length. There was a qualitative and quantitative change in proteins causing rapid loss of pollen viability	(Das et al., 2014)
Wheat (*Triticum aestivum* L.)	The short period of stress (5 days) before anthesis	Floret fertility was also decreased by pollen abnormalities	(Prasad and Djanaguiraman, 2014)
Rice *Oryza glaberrima* Steud., *Oryza sativa* L. ssp. Indica, *O. sativa* ssp. aus	5.6 -45.4 °C	The maximum temperature for 50% pollen growth was variable in each species	(Coast et al., 2015)
Sunflower (*Helianthus annuus* L.) Maize (*Zea mays* L.)	Sunflower reproductive stage (R1) Maize (early reproductive phase)	Breeding lines with lower abscisic acid accumulation ability tend to have higher pollen viability	(Rauf et al., 2015) unpublished

Table 2: *Effect of reproductive phase drought stress on pollen viability.*

Species	Stage of stress	Pollen viability	Reference
Maize (*Zea mays* L.)	Start of pollen shedding	Strong association between pollen availability and grains per cob	(Hall et al., 1982)
Chickpea (*Cicer arietinum* L.)	Terminal drought	Pollen viability reduced by 50%, pollen germination decreased 89% in-vitro Pollen germination decreased by 80% in-vivo	(Fang et al., 2010)
Rice (*Oryza sativa* L.)	Reproductive phase	Pollen viability and seed setting had strong association under stress	(Nguyen et al., 2009)
Rice (*Oryza sativa* L.)	Flowering stage	Pollen sterility wascaused by the reduced sugar contents	(Fu et al., 2011)
Pea (*Lytharus sativus* L.)	First flowering	Pollen viability, germination and number of pollen tube reaching the ovary reduced by 13%, 25% and 31%	(Gusmao et al., 2012)
Rice (*Oryza sativa* L.)	Early reproductive drought stress	Pollen quality reduced due to loss of pollen viability, pollen grain production and pollen load on stigma	(Saragih et al., 2013)
Sorghum (*Sorghum bicolor* L.)	Reproductive phase	Pollen fertility reduced by drought and germplasm was discriminated over drought	(Sakhi et al., 2014)

Moreover, the genotypes having lower pollen viability showed reduced sugary contents (Gusmao et al., 2012). Thus, drought susceptible genotypes were unable to mobilize their carbon sources to the developing anthers causing pollen sterility. Pollen sterility under drought stress was investigated at the molecular level which showed that susceptible genotypes had high expression of carbon starved anthers genes while tolerant genotypes showed high expression of sugar transporter and cell wall invertase genes (Li et al., 2015). Pollen viability has also been shown to be affected by the plant growth regulator such as

Table 3: *Impact of salinity on pollen viability.*

Species	Provision of stress	Impact	Reference
Rice (*Oryza sativa* L.)	50 mM NaCl	Pollen sterility was due to limitation of soluble carbohydrates to the primary and secondary panicles	(Abdullah et al., 2001)
Rice (*Oryza sativa* L.)	100 mM	Pollen viability reduced but also affected the stigma receptivity	(Khan and Abdullah, 2003)
Halophyte (*Plantago crassifolia* L.)	100 mM	Variability among the genotypes for pollen viability	(Boscaiu et al., 2005)
Tomato (*Lycopersicon esculentum* L.)	150 mM during inflorescence development	Soluble carbohydrate concentration increased in leaves and decreased in inflorescence. Na+ not accumulated in tapetum nor in pollen	(Ghanem et al., 2009)
Rice (*Oryza sativa* L.)	Mixture of saline-sodic soil with normal	Pollen germination rate was 34.4% and 15.5% under low and high salinity respectively	(Wang et al., 2010)
Rice (*Oryza sativa* L.)	100 mM NaCl at booting stage	Pollen viability was decreased by 83% in saline susceptible genotypes whereas 23% in salt resistant genotypes. 18 different types of proteins were involved in various process including anther wall remodelling	(Sarhadi et al. 2012)
Chickpea (*Cicer arietinum* L.)	40 mM and 160 mM	No impact of salinity on pollen viability and invitro pollengermination	(Turner et al.,2013)

Table 4: *Generation mean of 100-seed mass (g) and pollen sterility (PS %) under heat stress.*

Crosses/Genotypes	CM-207 (P1) × CM-631		CM-89 (P1) × CM-615(P2)		HA-407 (P1) × RFSS-88 (P2)	
	100-SM	PS%	100-SM	PS%	100-SM	PS%
Parent (P1)	5.19^{a}± 0.57	10.29^{c}± 8.58	5.56^{a}± 0.58	12.15^{c}± 8.23	6.34^{a}± 0.58	9.15^{c}± 6.39
Parent (P2)	1.64^{d}± 0.34	67.54^{a}± 9.03	1.67^{d}±0.56	71.54^{a}± 13.03	1.73^{c}± 0.58	68.89^{a}± 12.39
P1 × P2 (F1)	2.91^{c}± 0.22	51.77^{b}± 10.21	3.12^{c}± 0.55	56.23^{b}± 11.39	4.21^{b}±1.10	50.22^{b}± 10.87
P1 × P2 (F2)	4.31^{b}±2.14	54.21^{b}±18.41	3.86^{b}±2.12	61.49^{b}±19.35	4.55^{b}±2.19	52.39^{b}±21.22
Degree of dominance	-0.69	-0.44	-0.25	-0.48	0.07	-0.37
σ Phenotypic	2.15	338.94	4.09	314.29	3.32	297.28
σ Genotypic	1.75	248.62	3.75	197.23	2.72	161.41
Heritability	0.81	0.73	0.91	0.63	0.82	0.54

abscisic acid (ABA) (Table 5). The ABA is known to be involved in mobilization of photosynthates to roots causing starvation in the developing anthers. Ethylene has been documented that is involved in multiple stresses including drought (Stepanova et al., 2009), the interaction between ABA and ethylene through drought, bud dormancy and fruit ripening make the plant reaction to drought stress very complex (Pinheiro et al., 2010). Transcriptomic reaction to drought stress interact with numerous stress pathways and hormones that differ naturally in the time of day, moreover a set of 19 core genes were celebrated, containing a CIPK7, sulfate transporter, numerous PP2Cs and NF-YB transcription factor (Wilkins et al., 2010). Water stress studies inclusion with microarray exploration of poplar clones in two environments exhibit epigenetic regulation to the environment. Drought stress plants prompted ample responses for photoinhibition, defense against ROS and osmotic regulation (Raj et al., 2011). Microarray analysis of stress treatments on rice directed NAC transcription factor that stimulated the expression level of number of stress tolerant genes and increased the drought and salt tolerance in the field (Hu et al., 2006).

Salinity stress

Salinity stress was also known to affect the pollen viability. Studies have shown that reproductive phase salinity stress had caused significant pollen sterility but variation among and within species has been known to exist (Turner et al., 2013). Saline tolerant genotypes show the lesser degree of pollen sterility in comparison to the susceptible genotypes (Sarhadi et al., 2012). Therefore, pollen viability could be exploited a good criterion of salinity resistance in diverse crop species (Table 3). It has been noted that, salinity stress reduced the mobilization of soluble carbohydrates to the inflorescence causing the significant reduction in pollen viability (Ghanem et al., 2009). However, it was identified that accumulation of Na+ did not occur in the tapetum cells or anthers showing that reduction in pollen viability did not occur due to toxic effects of ions rather occurred due to lack of photosynthates mobilization for the inflorescence. Pollen morphology was also shown to change under salinity stress. It was shown that pollen of rice (*Oryza sativa* L.) obtained under salinity stress had more smooth surfaces and less visible curves when compared with pollen of non-stress condition (Wang et al., 2010). Salinity induced more responses in ions transport, energy metabolism and protein synthesis. A comparison experiment of short-term stresses specified that slow continuing stress was more complex than severe stress response (Dinneny, 2008), while metabolomics comparison study among salinity, drought, heat, light and low temperature stress detected metabolites that are vital in abiotic stresses, are stress specific (Cramer, 2007, De, 2005; Caldana, 2011). Transcription factors; AP37 and AP59 were functionally described, over-expression of AP37 enhanced salinity, drought and cold tolerance in rice (Oh et al., 2009). Vanderauwera et al. (2005) examined the role of H_2O_2 in salinity stress using microarray, hence reported that H_2O_2 was not only associated in signaling in salinity but also in high light stress.

Pollen viability assessment

Pollen viability can be indicated through various methods (Riano and Dafni, 2000). However, pollen viability and in-vitro germination have been extensively exploited to estimate pollen viability (Satish and Ravilumar, 2010). High crop yield also depends upon pollen viability and it has paramount significance in the hybridization programme (Patel and Mankad, 2014). Pollen quality can be estimated on the basis of vigour and fertility. Vigour refers to the pollen germination speed and rate of pollen tube germination. In vitro pollen germination tests have been used for the assessment of pollen germination percentage and vigour over time (Chatterjee et al., 2014; Sulusoglu and Cavusoglu, 2014). If pollen viability of genotypes is high, therefore; genotypes can be considered good pollinator and assessment of pollen fertility and germination potential are important criterions for pollen evaluation (Gaaliche et al., 2013). Moreover, pollen viability is also estimated after the induction of sterility through chemical hybridizing agents (Razzaq et al., 2015). Different stains such as pollen viability tests have been used in the past for the assessment of pollen viability and the relative estimate of fertilization potential (Huang, 2004; Ilgin et al., 2007; Frescura et al., 2012). The use of reliable methods for functional quality of pollen is important in the evaluation of pollen during storage, crop improvement, genetics and fertility studies, in the breeding of crops (Radicevic et al., 2013). Furthermore, there is the linear relation between pollen fertility and germination capacity in several fruit species. Stain tests were easy and fast but sometimes pollen germination methods were important to estimate pollen fertility (Sulusoglu and Cavusoglu, 2014). In vitro pollen germination provides quantitative measures, which were found to be lengthy procedures and can affect by sucrose%, temperature, hydration and existence and lack of ions. The studies indicated that, fresh pollen must analyse in minimum time after collection. The pollen viability tests that used in the past also have certain drawbacks including staining of viable and nonviable pollen grains. There were numerous methods of estimating pollen quality: tests based on pollen cell membranes, staining of pollen, in vitro pollen germination, pollen germination on stigma. However, neither of the tests could be optimize in all crops for the assessment of pollen viability (Dafni and Firmage, 2000).

Selection for the improved Pollen viability under abiotic stresses: identifying the knowledge gap

Pollen are good indicator of various abiotic stresses on plants and has been used to discriminate the crop germplasm against various stresses. Moreover, pollen viability has also shown the close association with the filled grain or grain number under stress conditions such as drought and heat stress (Kalyar et al., 2013). However, only a few studies have practically improved the pollen viability in the segregating generations through the selection of high pollen fertility per se. Information regarding the heritability of pollen viability is lacking. It has been known that high heritability in the segregating generation warrants good selection response. Moreover, magnitude and type of genetic variation associated with pollen viability also important in improving the trait and breeding procedure to be adapted for the improvement of trait per se (Kalyar et al., 2014). It has been known that traits having the high degree of additive variance could be exploited as a selection criterion in earlier segregating population and can be improved through simple selection procedures. In order to obtain information regarding the magnitude, type of genetic variation and heritability associated with pollen sterility. The pollen sterility in various generations of sunflower (*Helianthus annuus* L.) subjected to heat stress after staining the pollen with tetrazolium chloride (Kalyar et al., 2015). The parental plants were exposed to high day temperature of 45°C. The results have been presented in Table 4, it was shown that parental means significantly differed for each other and F1 means had significant lower pollen sterility than parents with high pollen sterility. Additive to partial dominance toward higher pollen viability was noted in the inheritance of these traits showing that traits could be improved through selection in segregating generation. Heritability for pollen sterility was moderate to high. There was a strong association between the plants of F2 for pollen fertility and filled grain%. The pollen sterility increased the number of unfilled grain. Thus, reducing the 100-seed masses of genotypes under heat stress (Kalyar et al., 2014).

On the other hand, *in vitro* pollen germination and tube growth of maize (*Zea mays* L.) showed high genetic variability and heritability under high heat stress. Traits were dissected with 8 RFLP markers in the RIL population which showed that five QTLs were linked with pollen germination and six QTLs were shown to be associated with pollen tube growth. It was also shown that both traits were controlled by the independent set of genes (Frova and Sari-Gorla, 1994). Similarly, population fitness was increased after exposing the segregating population to the natural heat stress cycle. It was noted that heat stress could only allow the heat-resistant pollen to germinate over the stigma resulting in the evolution of heat resistant plant types Kalyar et al. (2013).

Khuram (2015) made analyses to evaluate pollen viability in 95 genotypes including 19 parents and 76 crosses obtained from parents and their general combining ability (ability to produce superior progeny). Results showed that there was significant ($P \leq 0.05$) variation among the parents and crosses for pollen viability under temperature stress. Line × Tester analysis of pollen viability also showed that variation due to male and female parents was insignificant ($P \geq 0.05$). The relationship between the mean pollen viability of parents and general combining ability indicated that there was no relationship between these two variables (Figure 3). However, few breeding lines had pollen viability with good general combining ability. For instance, quadrate III was populated by breeding lines with "B-6" and "B-20" with good pollen viability and general combining ability under heat stress. Moreover, quadrate I was populated with breeding lines such as "B-2", "B-5" and "B-6" having high general combining ability with low pollen viability. Quadrate IV was occupied by breeding lines such as "B-14", "B- 21", "B-3" having low combining ability and higher mean values for general combing ability. Breeding lines in quadrate I and IV should be crossed to develop transgressive segregants. However, the trait could be used as the dominant marker for discriminating sunflower advanced lines under heat stress.

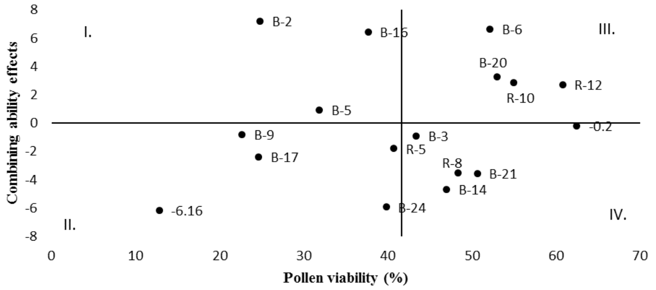

Figure 3: *The relationship between the general combining ability and pollen viability of parents in sunflower (Helianthus annuus L.)*

Abscisic acid (ABA) is a common stress signal that is produced in response to various abiotic stresses such as extreme temperature, drought and salinity (Shinozaki et al., 2000; Tuteja, 2007; Fujita et al., 2011). ABA signals, which are generated at various growth stages of plants were known to improve the stress adaptability and survival of crop species and ABA over-expression was related with a reduction in leaf expansion rate and translocation of photosynthates to lateral roots at the expense of aboveground biomass in model species such as maize and *Arabidopsis* (Sharp et al., 2004; van Houtte et al., 2013). ABA also tends to reduce the reserve mobilization to reproductive organs. Moreover, ABA production is antagonistic to cytokinins and a high ABA concentration increases pollen sterility in maize (Niazi et al., 2014). Sunflower and Maize plants with low ABA accumulation under various abiotic stresses such as drought, salinity and heat stress were selected from somaclonal populations (R3) and subsequently analysed for pollen viability among 10 progenies developed from the selection of the plants with high or low ABA (Table 5). The genotypes with higher (ABA) tended to perform poorly with lower pollen viability while genotypes showing lower ABA contents in their leaves showedhigher mean values under three abiotic stresses (Table 5).

Table 5: *Performance of regenerated (R$_3$) population of sunflower and maize breeding lines from drought stress (DS), heat stress (HS), drought × heat stress (DS × HS) and salinity stress (SS). Traits evaluated: abscisic acid (ABA) content and pollen fertility. Values were averaged over five breeding lines of sunflower and maize.*

Cell lines	**ABA (ng FW^{-1})**					**Pollen fertility (%)**				
	Control	DS	HS	DS × HS	SS	Control	DS	HS	D × HS	SS
Sunflower										
High accumulator	18.34g ± 2.12	58.87b± 3.36	46.39d ±2.23	54.31c ±1.53	64.38a ±5.11	96.67a ±1.52	41.52e± 2.38	29.37g± 5.17	23.46 h± 3.36	36.25f ±4.62
Lowaccumulator	16.72g± 4.29	26.34ef ± 2.19	19.57 g± 1.93	23.29 f ±1.27	29.23 e ±5.22	95.74 a ±1.37	67.29 b± 1.97	61.34 c ±5.37	57.36 d± 2.94	63.28 c ±4.12
Maize										
High accumulator	27.56 e ±3.42	74.27 b± 4.57	80.81 a ±1.94	77.21 b± 1.62	76.54 b± 4.22	97.82 a ±2.55	36.42 g± 2.89	24.52 h± 2.16	19.34 i ±4.81	40.37 f ±2.72
Low accumulator	32.28 d± 3.69	47.55 c ±3.68	34.42d± 2.33	46.48 c ±2.14	45.33 c ±24	98.16 a ±2.33	71.39 b± 3.33	68.29 c ±5.58	58.22 e ±6.27	66.38 d± 4.52

** means within rows and columns of sunflower or maize with the same letter are statistically similar ($P \geq 0.05$; LSD multiple comparison test). Breeding lines mean was averaged over ABA concentrations. ABA was determined through Blagoeva et al. (2004) and pollen fertility was determined by fixing pollen 2% triphenyl tetrazolium chloride early in the morning (8:00 a.m.).*

Table 6: *Performance of regenerated (R3) population from drought stress (DS), heat stress (HS), drought × heat stress (DS × HS) and salinity stress (SS). Traits evaluated: leaf area (cm^{-2}) and a number of grains per head or cob-1 (sunflower and maize, respectively). Values were averaged over five R$_3$ lines of sunflower and maize.*

R3 lines	**No. seed head^{-1} (sunflower); No. grains cob^{-1} (maize)**				
	Control	DS	HS	DS × HS	SS
Sunflower					
High accumulator	159.28 a±22.54	92.34 d±26.22	111.37f±23.26	56.42 e±13.61	89.62 d± 14.38
Low accumulator	164.46 a ±16.61	136.53 b± 16.54	138.24b±15.34	119.47c±23.58	121.38 c± 9.34
Maize					
High accumulator	235.78 b ±18.97	88.35 f ±21.16	56.37 g ± 9.27	51.38 g ±16.47	79.31 f ±15.66
Low accumulator	256.22 a± 19.35	186.42 c ±15.37	121.29 d± 14.32	116.73 e ±13.57	168.48 e ±9.22

** means within rows and columns of sunflower or maize with the same letter are statistically similar ($P \geq 0.05$; LSD multiple comparison).*

In comparison to various abiotic stresses, heat stress showed the highest repressing effects on pollen fertility in both species followed by salinity stress in sunflower species and drought stress in maize specie. The results showed that selection for lower ABA contents could be exploited to improve the pollen viability. It also increased the grain number in sunflower and maize species showing that increasing pollen fertility could also increase the grain number under different stresses (Table 6).

Methods for improving pollen viability

A patent invention about the method for improving pollen viability and consequences increasing the number of more fertile flowers by the treatment of seed plants or that locus where plants are intended to grow with the strobilurin fungicide. So, this invention also related that to treat seed-producing plants for increasing fertile flowers with at least one of the strobilurin group fungicide, specifically pyraclostrobin. Fungicide could be accomplished in many ways; may apply directly to the seeds or in the soil, where the seed is to be planted (example in-furrow application). Instead, it may be applied after germination as a foliar application. In cereals particularly maize, more preferably treated during v6 to v8 growth stages (Mendieta and Granados, 2015). Furthermore, another study directed to identify the effect of boron on rice reproductive growth, pollen viability, stigma receptivity and yield attributes. Results showed that mean pollen viability of 86% and stigma receptivity was recorded the almost alike pattern of pollen viability at 0.4 ppm. A higher number of spikelets and grain number were recorded at 0.8 ppm and 0.4 ppm respectively of boron spray. Mean maximum rice grain yield was recorded at 0.4 ppm boron spray; this increased yield was mainly due to increased pollen viability and stigma receptivity (Guru et al., 2016). In these greater cited studies aimed to determine the effect of nitrogen and phosphorus respectively on pollen production and performance in *Cucurbita pepo* (Cucurbitaceae). Nitrogen treatments affected the mean size and number of pollen grains per staminate flower but not affected the mean total number of staminate flowers per plant. Pollen developed by the high nitrogen treatment plants, commercial recommended dose produced more percentage of seeds and fertilized the ovules with faster-growing pollen tubes. On the other hand, phosphorous treatments had the significant effect on female and male reproductive output. Pollen developed from the high phosphorous treatment plants, commercial recommended dose, and they produced more seeds, in contrast to low treatment soils. However, evaluated that heterogeneity of the soil nitrogen and phosphorous can significantly affect seeds percentage due to the differences in pollen performance (Lau et al., 1993, 1994).

Management of heat stress

Crops yield is the consequence of the genotypes expression and their interaction with the environment (G×E). Agriculture is the complex field; crop management also has a significant importance that leads to the G × E × M model. At the farming level management approaches could be adopt; planting dates can be adjusted to avoid crops from heat stress (Olesen and Bindi, 2002; Lotze-Campen and Schellnhuber, 2009). Crops planting and harvesting dates from around the globe have been noted in so-called crop calendar. This calendar comprised ~ 1300 planting and harvesting dates data about 19 crops, delivers the relative estimation of the time at different geographic locations (Sacks et al., 2010). Secondly, the enhancement by water management that can lessen heat stress, as due to transpiration temperature of foliage remains control (Smithers and Blay-Palmer, 2001). Thirdly, the strategy is by changing the site of crop cultivation, to grow in cooler regions. This has been happened in several crops including; rice and maize (Tchebakova et al., 2011; Park et al., 2012).

In the breeding for resistance to heat stress, the evaluation of breeding lines must be in hot areas for the selection of thermotolerance traits (Mickelbart et al., 2015). Conventional breeding has been successfully developed heat-tolerant varieties in crops, and recurrent selection effectively improved wheat yield by T. *tauschii* as a donor of the genes that improved larger grains and grain filling (Gororo et al., 2002). Conventional breeding successfully improved a number of crops and new varieties also developed, however physiological and genetic basis remained unclear. Hence, the supplement of marker-assisted selection could evaluate germplasm for better heat tolerance and effective breeding of the complex traits through genomic selection (Paran and Van Der Knaap, 2007). Heat tolerance is polygenic trait, so for the tolerance at genetic level experiments have been done in several crops to identify QTLs that are linked to heat tolerance and its related traits in a number of crops, includes *Arabidopsis*, brassica, rice, wheat, sorghum, barley and cowpea (Wahid et al., 2007; Collins et al., 2008; Ainsworth and Ort, 2010; Jha et al., 2014). In potato 9 QTLs about inside heat necrosis in tubers, in maize 5 and 6 QTLs related to pollen quality and its tube growth with high heritability 0.64 and 0.69 respectively. In tomato 6 QTLs related to a high temperature having more than 33%, phenotypic variation has been identified. A new study about rice spikelet fertility under heat stress and confirmed the recessive QTL at chromosome number 4, that shares 15% more spikelet fertility have been discovered. QTLs can be effectively introduced into the crossable breeding germplasm beside transgenic approaches (McCord et al., 2011; Ye et al., 2015).

The identification and introgression of superior wild alleles, those are deficient in cultivated crops have been a great interest. For example, the introgressions of wild alleles in several cereal crops such as wheat, rice and maize have been a source of novel alleles (Grandillo et al., 2007; Feuillet et al., 2008; Pradhan et al., 2012). Genes transcriptional profiling has been completed in several crops including tomato, rice, brassica, grapes and barley, about the stressed, non-stressed and between heat susceptible and tolerant variants (Frank et al., 2009; Frey et al., 2015; Liu et al., 2012; Dong et al., 2015) In an extensive study, the plant's signal transduction pathways were studied there were reprogramed due to heat stress and proteins are related to metabolism in a specific manner. However, this study was completed in different crops and revealed substantially relationship with stress-responsive genes. Due to plants exposure to heat stress, reactive oxygen species (ROS) developed in cellular compartments in several aerobic metabolic pathways (Miller et al., 2009; Chou et al., 2012; Volkov et al., 2006; Mostofa et al., 2013; Bokszczanin et al., 2013).

To prevent cell damage in the response to stress, ROS scavenging machinery is regulated by genes that are important in thermotolerance (Lillig et al., 2008). In response to heat stress, the best mechanisms are the formation of heat shock proteins (Wang et al., 2004). Due to experiments in rice, tobacco, tomato and *Arabidopsis* have revealed that greater thermotolerance can be achieved by the overexpression of heat shock transcription factors and heat shock proteins (Grover et al., 2013).

Conclusions and Recommendations

This study highlights that, pollen viability is an important index of abiotic stresses tolerance. These abiotic stresses affect plants normal growth and development in serious yield losses. Reproductive is considered the developmental phase in crop plants that most susceptible to abiotic stresses especially heat stress due to increased temperature. Sustainable crop production is under threat from the considerable rise in world temperature. However, the developments of crop varieties resistant to heat stress are most important to sustain crop yield. A number of studies are available on the mechanism of heat resistance in crop plants, only little direct methods could possible to improve heat resistance in crops. Therefore, during crop breeding for heat resistance the evaluation of breeding lines must be in hot areas for the selection of thermotolerance traits and selection criteria is the most important that also vary crop to crop. Few general methods of improving pollen viability have been included in this review article. Tolerance in new crop cultivars is restricted due to the frequently narrow genetic base. Exploration the potential of wild relatives and landraces and their exploitation in crop improvement by conventional and next-generation breeding with clarification of the cellular, molecular and biochemical mechanisms about the parameters of fertility will increase yield and quality of several agricultural valued crops. Furthermore, due to the complex nature of the tolerance against abiotic stresses and its concern with the climate change, it is indispensable to face this breeding challenge in multi-disciplinary complete approach by international collaboration, including supports of the government, research and academic institutions.

Authors Contribution

MKR, SR and MK wrote the paper. SI conceived and designed the pollen viability experiment. JAB and AF analyzed the data. AR data collection. GX and JG critically review the paper at the end.

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