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Understanding the pollen and ovule characters and fruit set of fruit crops in relation to temperature and genotype – a review

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Summary

Plant reproduction is indispensable for maintaining crop productivity and species sustainability which basically involves pollen and ovule. Productivity is influenced by temperature stress in various fruit crops worldwide and important factor for unfruitfulness is failure in fertilization. Understanding of the plant reproductive characters in relation to genotype differentiated response across different temperatures will help to develop strategies in overcoming temperature stress. Available literature suggests that variation in pollen viability, germination and tube growth and ovule normality exists among different fruit species, cultivars and flower types (even in the same plant as in litchi) and are strongly influenced by temperature stress and suitable temperature for proper pollen and ovule performance has been identified for various fruit crops. Possible reasons to explain the mechanism for impairment of proper functioning of pollens and ovule are discussed. Further, low and erratic fruit set, improper fruit development and stenocarpic fruits as a result of temperature stress leads to reduced yield. We suggest considering *in vitro* performance for establishing *in vivo* nutritional requirements and standardizing critical leaf nutrients by way of correlating with pollen germination, tube growth and ovule normality per cent. Male and female parents can be identified for breeding purpose and region specific adoption based on temperature prevalence in the area.

Introduction

Climate change is the defining challenge for human development and ecological well being in the 21st century and increase in average global temperature of between 1.1 and 6.0 °C by the end of this century is predicted by the Intergovernmental Panel on Climate Change (IPCC). The consequence of 2 °C temperature rise is grave for potentially millions of people through death, injury and dislocation from flooding, fire and diseases, adverse affects on water quality, species extinction and reduced agricultural yields. To which extent temperature stress will influence plant survival and crop yield is critical in evolutionary, environmental, economic and social terms. Global warming may represent another challenge to plant productivity and geographic distribution (HEDHLY et al., 2009) and tolerance to temperature stress is becoming a desirable trait. Understanding of temperature stress physiology during plant reproductive process is still incomplete. A number of reproductive processes must occur in a highly concerted fashion for fertilization to occur and pollen development and function may be the most thermosensitive reproductive processes to high temperature (HEDHLY et al., 2009). The yield of plant species with reproductive structures of agricultural importance is exceptionally sensitive to temperature stress during flowering (ZINN et al., 2010). Heat stress can limit fertilization by inhibiting male and female gametophyte development, pollen germination and pollen tube growth (HEDHLY, 2011).

However, moderately low temperature also reduces pollen performance and could result in dysfunctions of the fertilization processes given the short stigma receptivity intervals. Male gametophyte development is especially sensitive to temperature both at pre- and post-pollination levels. Pollination of flower is necessary to achieve a satisfactory fruit yield and improper pollination factors can drastically influence productivity and have serious economic impacts. Since, successful pollination will be affected by pollen viability, germination and tube growth, impairment of these functions and qualities could threaten species reproduction and survival. Also, the maternal tissues of the pistil and the female gametophyte, traditionally considered more tolerant, have also been reported to be sensitive to temperature stress. Above these individual responses, temperature stress might lead to developmental asynchrony in pollen-pistil-ovule functioning leading to reduced fertilization levels (HEDHLY, 2011). Thus, studies on the effect of temperature stress on reproductive process may be of great importance for choosing the appropriate variety for a certain area according to the microclimate and to elucidate the correlation between low fruit set and incidents of extreme temperature preceding and during flowering period. Strong genotype-differentiated response in low, high and moderate high temperature stress could be exploited by plant breeders towards producing new temperature tolerant varieties.

Effect of temperature on pollen characters of different fruit species

Air temperature is considered as the main environmental factor affecting the pollination and fertilization progress and was evident in sweet cherry (ZHAO et al., 2008). Studies on the influence of genotype, temperature, nutrient media (types and sources, concentration, pH) on pollen germination and tube growth has been established in many crops both *in vivo* and *in vitro* though open field conditions are more susceptible to abiotic stress since they are directly exposed at environmental extreme. The effects of temperature on pollen germination and pollen tube growth have earlier been reported by several workers in various fruit species (MELLENTHIN et al., 1972; LUZA et al., 1987; CEROVIC and RUZIC, 1992A; EGEA et al., 1992).

Pollen viability and germination

Temperatures above the optimum can exert negative effects on plant reproductive development. A pronounced detrimental effect of high day/night (D/N) temperature regimes 27/22 °C and particularly 32/27 °C during floral development on pollen viability of litchi has been reported by (STERN et al., 1996). The litchi originated in Southern China and its center of cultivation is the Ghuangzhou region, where temperatures during floral development (February and March) are ≈ 20/10 °C (GROFF, 1921). Optimum temperature for some reproductive processes might reflect, indeed, species origin or adaptation. For example, while for many species native to or cultivated in temperate regions optimum temperatures in the range 15-25 °C are usually recorded [examples include sweet cherry at 25 °C (WEINBAUN et al., 1984), or almond (*Prunus dulcis* Mill.) at 16 °C and peach at 23 °C (LEWIS, 1942). These adaptations can be best seen in natural populations that flower during extremely high or low temperatures.

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The rate of pollen viability in walnut was high (> 75 %) for all the cultivars studied (Mert, 2009). Similarly pollen viability rates of 32 different walnut cultivars were reported to vary between 81 % and 94 % (Sutyemez, 2007). In another study pollen viability ratio of selected 19 walnut cultivars varied between 77-92 % (Sutyemez and Eti, 2006). In some previous studies, similar results for pollen viability were reported in hazelnut (49-97 %) (Beyhan and Odabas, 1995), chestnut (8.8-35.8 %) (Beyhan and Serdar, 2009), apricot (76-86 %), sweet cherry (67-81 %), and sour cherry (71 %) (Bolat and Pirlak, 1999). It was observed that no germination of pollen from any of the *Juglans regia* (Persian walnut) or *Juglans nigra* (black walnut) cultivars occurred below 14 °C and maximum germination was obtained at 28 °C in *J. regia* and 32 °C in *J. nigra* (Luza et al., 1987). Temperature had significant effect on the germination percentage of different walnut cutivars ('Şebin', 'Kaplan 86', 'Yalova 3', 'Pedro', 'Hartley' and 'Franquette') (Mert, 2009) and pollen germination rates increased significantly with increasing temperatures with highest germination rates at 27±1 °C temperature in both years (26.94-73.98 % and 22.78-70.86 %, respectively) of study while, in cv. 'Yunxin' the optimum temperature for pollen germination was recorded at 25 °C (Wu et al., 2008).

Usually, pollen germination is negatively affected by high or low ambient temperatures (Westwood, 1978). In almond, pollen germination was observed at temperatures between 4.4 and 10 °C (Griggs, 1975). Optimum temperature required for pollen germination in apricot was about 15 °C and 20 °C (Pirlak, 2002). Pollen germination (Egea et al., 1992; Pirlak, 2002) and tube growth speed was low at 5 °C for apricot varieties and the germination rate increased with increasing temperature but temperatures above 25 °C caused a decrease in pollen germination rates (Egea et al., 1992). Similarly, investigation of pollen germination and pollen tube growth rate of different sweet cherry varieties at different temperatures (5, 10, 15 and 20 °C) showed that pollen germination was low at 5 °C and was optimum at 15 and 20 °C (Pirlak, 2002). In cherimoya, 20-25 °C was found optimum for pollen germination, while germination decreased at 30 and 35 °C; and at 10 °C, only 1.8 % of the pollen grains germinated (Rosell et al., 1999).

In vitro pollen germination of olive cultivars Picudo, Picual, Manzanila, Hojiblanca and Gordal decreased at 35 °C (Fernandez-Escobar, 1983) and reduced germination at 30 °C has also been reported for 'Manzanillo' olive (Cuevas et al., 1994) and was concluded that optimum temperature for pollen germination was 20-25 °C while pollen of 'Amigdalolia' olive germinated similarly at 25 and 30 °C (Fernandez-Escobar, 1983; Cuevas et al., 1994). Even a short-term exposure at high temperature extremes (40 °C) combined with low RH (20 %) prior *in vitro* culture had detrimental effect on pollen performance of four olive cultivars viz., Koroneiki, Mastoidis, Amigdalolia and Kalamata. Pollen culture at 15 °C resulted in the lowest germination and tube growth rates and low temperature pre-incubation treatment (10 °C) showed no constant effect on pollen germination (Koubouras et al., 2009). Pear (Vasilakakis and Porlingis, 1985) and avocado (Loupassaki et al., 1997) pollens germinated best at 25 °C and reduction in pollen germination occurred due to heat stress. Pollen germination of different Pistacia genotypes ranged from 83 % to 97 % (Kamiab et al., 2007). In strawberry cv. 'Tochiotome' both viability and germination were not significantly different between two day/night temperature (32/27 °C and 27/22 °C) regimes. Approximately 90-93 % of ovules were fertilized at 27/22 °C however; the fertilized percentage of ovules at 32/27 °C was 52 % to 85 % depending on the inflorescence type. Embryo development in both inflorescences was accelerated at 32/27 ºC (Pipattanawong et al., 2009).

In mango, optimum temperature was found to be 15 and 33 °C (Issarakraisila and Considine, 1994) and 15-25 °C (Sukhbivul et al., 2000) and at 10 and 30 °C pollen germination was reduced and that 25 °C was optimum for *in vivo* incubation (Sukhbivul et al., 2000). The optimum temperature for pollen germination of citrus *in vitro* was of 25 °C, while pollen germination under different *in vitro* incubation temperatures, showed a progressive increase in germination rate from 15 °C to 25 °C, with a sharp decrease when temperature reached 30 °C (Distefano et al., 2012). Furthermore, temperature appears to have an effect on self-incompatibility reaction by affecting the place where pollen tubes are arrested. Absence of germination at 10 °C, and the optimum germination temperature of 25 °C reflect the subtropical origins of all *Citrus* species examined. The critical low temperatures for the induction of pollen sterility in lemon is 18 °C (Soost et al., 1975), respectively and low temperature in the early spring may be an obstacle for the pollen germination and pollination. Hence, temperature extremes either low or high can be detrimental or may lead to impairment of pollens.

Pollen tube growth

Pollen tube growth rate was evaluated under increasing temperatures, in *Datura stramonium* (Buchholz and Blakeslee, 1927) where pollen tube growth rate increased linearly by a factor of 4.5 from 11 ºC to 33 ºC (from 1.28 mm/h to 5.86 mm/h). Since then information on temperature affects to pollen tube growth rate, built up confirming this fact in different fruit species (Mellenthin et al., 1972; Lombard et al., 1927; Socias i Company, 1976). This response pattern of pollen tube growth with temperature seems to be a general phenomenon since it has been confirmed in many seed plants, and mathematical models for pollen tube growth in relation to temperature have been developed (Jeffries et al., 1982). This variation in the rate of pollen tube growth was suggested to be the result of increased metabolism at higher temperatures, typical for most biological growth rates (Weinbaum et al., 1987). It is now conclusively proved that temperature at bloom is one of the main factors affecting fruit set (Mellenthin et al., 1972; Vasilakakis and Porlingis, 1972). Since high temperatures accelerate and low temperatures retard pollen tube growth rate, it would be expected that fertilisation and, hence, fruit set would be enhanced by moderately high temperatures and reduced by low temperatures during bloom. However, this is not always the case, due to the fact that temperature also does have an effect on pistil development, accelerating development at high temperatures and slowing it down at low temperatures. Consequently, high temperatures during flowering accelerate pollen tube growth, but also maturation and hence early degeneration of the stigma (Burgos et al., 1991) and ovule development (Postweiler et al., 1985; Hedhly et al., 2004). Temperature around 25 ºC was found most favourable to accelerate *in vivo* pollen tube growth, to advance fertilization and to obtain a good initial fruit set in olive (Cuevas et al., 1994). In avocado cultivars, pollen tube length further increased though pollen germination decreased after *in vitro* culture at 30 ºC. An increase in temperature also reduced pollen germination but accelerated pollen tube growth in sweet cherry (Hedhly et al., 2004). Pollen tube growth rate of 'Manchurian' crabapples and 'Golden Delicious' apple in the style increased quadratically with increasing temperature from 13 to 29 °C. Pollen tube growth rate in the style increased with increasing day/night temperature from 7/0 to 24/7 °C. The time required for pollen tubes to grow to the base of styles decreased with increasing day/night temperature from 13/2 to 24/7 °C (Yoder et al., 2009). In *in vivo* studies with litchi (Stern and Gazit, 1998), it was observed that high temperature regime (32/27 °C) had a pronounced detrimental effect on further pollen tube growth after reaching the base of the style. Pollen tubes reached the ovary in ≈20 % of the flowers and in no flower did they reach the ovule. Variation in the pollen tube growth was observed at different temperature according to cultivars with decline in tube growth at temperatures in excess of the optimum (Irenaeus, 2012).

The pollen tube growth of olive cultivars Picudo, Picual, Manzanila, Hojiblanca and Gordal were found to decrease at 35°C (FERNANDEZ-ESCOBAR et al., 1983). High temperatures in excess of the optimum (30 °C) resulted in an increase in the rate of pollen tube growth through the style for *Prunus avium*, but decreased the number of pollen tubes to reach the base of the style (HEDHLY et al., 2004). Pollen tube length increased with increasing incubation temperature and the longest pollen tube were measured at 25 °C in sweet cherry (KOYUNCU, 2006) while, in citrus, the most favourable temperature to accelerate *in vivo* pollen tube growth depended on the particular male female interaction and ranged between 15 and 25 °C (DISTEFANO et al., 2012).

On the other hand, low temperatures have also been reported to slow pollen tube growth in pear (MELLINTHIN et al., 1972; LOMBARD et al., 1972), almond (SOCIAS I COMPANY et al., 1976), plum (JEFFRIES et al., 1982), sweet cherry (GUERRERO-PRIETO et al., 1985) and apple (WILLIAMS et al., 1970). As a general rule, low temperature during flowering slow pollen tube growth rate but extend the effective pollination period (EPP) through an ovule life-extension effect (VASILAKAKIS et al., 1985; TROMP and BORSBOOM, 1994). However, extreme low temperatures can shorten the EPP if longevity of the ovule does not outweigh the slower growth rate of pollen tubes (LOMBARD et al., 1972). High temperatures, while increasing pollen tube growth rate, shorten the EPP by shortening both stigma and ovule receptivity. Under the cold regime (17/22 °C), pollen tubes of litchi reached the ovary in all the flowers but did not proceed any further and no pollen tube reached the ovule reflecting a slow growth rate at this cold regime. Under the cool (22/17 °C) and warm (27/22 °C) regimes, tubes reached the ovule in ≈35 % of the flowers (STERN and GAZIT, 1998). The virtual absence of sexual reproduction of 'Tainong 1' mango at low temperatures appears to be largely due to slow growth of pollen tube *in vivo* and to a low rate of successful fertilization (HUANG et al., 2010). However in olive, low temperature pre-incubation treatment (10 °C) had positive influence on tube length in four cultivars (KOUBOURIS et al., 2009).

Mechanisms leading to pollen impairment due to temperature stress

Report explaining the possible mechanism of heat stress leading to poor quality and performance of reproductive organs on specific fruit crop is scarce but can be explained from the evidences available in other plants. Heat stress adversely affects pollen meiosis and germination, ovule development and viability and development of the embryo (PEET et al., 1988). At high temperatures decreased pollen production may be related to anther indehiscence (PORCH and JAHN, 2001) and lower pollen viability could be related to decreased carbohydrate metabolism (DATTA et al., 2001; KARNI and ALONI, 2002), all of which could significantly influence nourishment of pollen mother cells which could lead to infertile pollen. The effect of heat stress on pollen viability was associated with carbohydrate metabolism during anther development (PRESSMAN et al., 2002). Under optimal temperature soluble sugar concentration gradually increased in pollen. Continuous high temperature prevented the increase in starch concentration and led to decrease soluble sugar in mature pollen. These possibly cause to decrease pollen viability.

Carbohydrates are the major nutrients which support pollen development (PACINI, 1996), whereas for germination of pollen grains, simple sugars are the principal metabolic substances (STANLEY, 1971). Anthers have the highest sink strength in the flower, and the large amounts of sugars are mobilized to the anthers to support early development (CASTRO and CLEMENT, 2007). It was reported that male sterility could be induced in tobacco by metabolic engineering of the carbohydrate supply to the developing pollen (GOETZ et al., 2001). Major alterations in gene expression under high temperature stress have been shown, paralleling tapetum degeneration and pollen sterility in rice (ENDO et al., 2009). Among these genes, enzymes involved in carbohydrate metabolism (e.g. cell wall and vacuolar invertase, sucrose synthase) and transport, are gaining higher research interest as indicators of losses in pollen viability due to temperature fluctuations. Both cold (OLIVER et al., 2005) and heat stress (PRESSMAN et al., 2005) have been shown to down regulate gene expression of several invertase and sucrose synthase isomorphs, and this inhibition was accompanied by a disruption of sucrose and starch turnover in developing pollen grains, and, hence, lower accumulation of soluble carbohydrates (OLIVER et al., 2005; JAIN et al., 2007). A complete understanding of temperature stress on pollen development must await further understanding of carbohydrate turnover during this phase. Interestingly, in a recent study in barley and Arabidopsis, it was observed that high temperature stress reduced endogenous synthesis of auxin in developing anthers, an effect that was suggested to be related to pollen sterility since exogenously applied auxin restored fertility (SAKATA et al., 2010). It may be concluded that continuous high temperature reduced the total number of pollens, viability and germination.

In a study in mango, it was observed that microsporogenesis is sensitive to low and high temperature stress and the formation of fertile pollen require temperature within the range of 10-35 °C. The developmental phase from meiosis to the pre-vacuolate microspore was found to be the most temperature sensitive phase of pollen development, though sensitivity continued through the end of vacuolation and began in the late microsporocyte phase. The critical temperature for the formation of fertile pollen in the mango cv. 'Kensington' seems to be about 10 °C. A night temperature below 10 °C (12 h regime) during meiosis resulted in pollen grains with low viability (<50 %). At the vacuolate stage, a night temperature of 9 °C (8 h) did not lower the percentage of pollen viability, but the viability of mature pollen was markedly reduced if the exposure to 9 °C lasts for 12 hours (ISSARAKRAISILA and CONSIDINE, 1994). Low temperature during flower development has been found responsible for the occurrence of defective reproductive organs in different fruit crops like almond (EGEA and BURGOS, 1995), grape (EBADI et al., 1995) and persimmon (FUKUI et al., 1995). Chilling injury has been implicated in damage to mango pollen (ISSARAKRAISILA and CONSIDINE, 1994) and probably also to mango female organs (ISSARAKRAISILA et al., 1992) precise aspect of pollen development which fails under low temperature stress is unclear. Meiosis is reported to be the stage of greatest sensitivity to low temperature in sorghum (BROOKING et al., 1976). Previous analyses have associated a depression of respiratory activity in anthers during meiosis with loss of viability (TORIYANA and HINATA, 1984). Early abnormalities in rice anthers as a result of cooling at the young microspore stage included increased content of non-reducing sugars and starch, decreased inorganic phosphates and acid phosphatase activity and dilation of the tapetum. These responses were followed by degeneration of the microspores (NISHIYAMA, 1984).

Natural low temperatures significantly affected pistil and male gametophyte development, resulting in pollen grains with low viability of mango. Meiotic chromosomal irregularities, including univalents, multivalents, laggards, bridges and micronuclei were detected at higher incidences and significantly greater proportions of nucleolus fragmentation and dissolution were detected when temperatures were low. Pollen tube growth was retarded under low temperature stress either *in vivo* or *in vitro* (HUANG et al., 2010). Pollen numbers and germinability were less in low night temperature (LNT) of 10±2 °C and day temperature which did not exceed 24 °C and suggested that low temperatures hinder pollen functioning in pepper, by interfering with starch accumulation at four days before anthesis, thereby decreasing the concentrations of soluble sugars in the mature pollen grains (SHAKED et al., 2004).

On the other hand, high temperatures impairment of mature pollen grains functioning was associated with a significantly higher sucrose and starch concentrations at four days before anthesis (as observed in pepper) (ALONI et al., 2001) and that sucrose accumulated due to a reduction in its utilization and that starch degradation rather than biosynthesis was suppressed under high temperatures. These contradicting results may suggest a different mode of carbohydrate metabolism disturbances by different stresses or cultivar specifity. Based on the demonstration (SHEORAN and SAINI, 1996) where levels of both reducing and non-reducing sugars in whole rice anthers increased substantially during the period of water stress, a suggestion (SAINI, 1997) that accumulation of sugars could be the result of disturbances in carbohydrate metabolism or of an inhibition of sugar utilization was made. Inhibition of sugar utilization could be relevant to the higher concentration of either sucrose or reducing sugars at four days before anthesis of the pollen developed under low night temperature (SHAKED et al., 2004). At that stage, starch concentration was lower than that in the pollen grains at higher temperature (night temperature of 20±2 °C and similar day temperatures) and all other processes were probably slower.

Actin cytoskeleton alteration is also an early signal component of pollen in response to low temperature (LT) stress and actin cytoskeleton alteration may be one of the reasons for inhibition of pollen germination and pollen tube growth of pear (WU et al., 2012). Depolymerization of the actin cytoskeleton in pollen will induce activation of pollen plasma membrane Ca^{2+} channels (WANG et al., 2004) and pollen tube growth prevention similar to self incompatibility as seen in pear (LIU et al., 2007). Identification of similar characteristics of low temperature induced depolymerization of the pear pollen actin cytoskleton $[Ca^{2+}]_{cyt}$ increase, and activation of pollen plasma membrane inward Ca^{2+} and outward K^+ channels was reported (WU et al., 2012). The activation of Ca^{2+} channels was mediated by the actin cytoskeleton structure. Low temperatures also induced intracellular Ca^{2+} influx. Moreover, the low temperature-elicited outward K^+ current was mediated by increased $[Ca^{2+}]_{cyt}$. When extracellular Ca^{2+} influx was chelated by EGTA, the LT-induced outward K^+ conductance decreased, indicating mediation by $[Ca^{2+}]_{cyt}$ These results were consistent with the outward K^+ channel being activated by $[Ca^{2+}]_{cyt}$ suggesting that activation of outward K^+ channels were the downstream targets of $[Ca^{2+}]_{cyt}$ increase in LT signal transduction.

Outward K^+ conductance has been identified in pollen and pollen tubes for several years (FAN et al., 2003). Ca^{2+}-activated outward K^+ channel in pear pollen tubes could be stimulated by depolarization voltage and reciprocally regulated by heme and its catabolism product, carbon monoxide. The carbon monoxide-induced K^+ outward flux largely inhibited pear pollen tube growth (WU et al., 2007). Thus, excess K^+ efflux can also hamper pollen tube growth. The cytoskeleton is an important factor regulating numerous cellular processes in plants (HIGAKI et al., 2007) such as a track for cytoplasmic streaming and organelle movement (TOMINAGA et al., 2000), positioning of organelles such as nuclei (KETELAAR et al., 2002), and maintenance of cellular architecture (TOMINAGA et al., 2000).

Role of polyamines

Among common polyamines (PAs), some studies have shown that cellular free putrescine (Put) was the most abundant polyamine followed by spermidine (Spd) and spermine (Spm), which were less abundant as seen in *Vitis vinifera* L. (FAURE et al., 1991) and carrot (FEIRER et al., 1994). However, this difference in polyamines predominance cannot be generalized, but might depend on species, the developmental stage, the duration of treatments, or the physiological status of cell lines as well as the media examined (EL MESKAOUI and TREMBALY, 2009). Several lines of evidence have suggested that PAs play a role in pollen germination and tube growth. In apple, PAs are synthesized during pollen germination (BAGNI et al., 1981). Both stimulation and inhibition of pollen germination and pollen tube growth *in vitro* by the addition of various concentrations of PAs in various apple cultivars have been demonstrated (XU et al., 1999). However, the lower and higher concentrations of the PAs were less efficient than an optimum concentration. Few reports (XU et al., 1999; PRAKASH et al., 1988) are available in the literature concerning PA synthesis inhibitor methyoxal-bis(guanylhydrazone) (MGBG) and pollen germination and pollen tube growth. Pollen germination to different degrees in two apple cultivars was inhibited by MGBG and at higher concentrations of MGBG, germination was completely inhibited in both cultivars (XU et al., 1999).

Increasing the temperature, particularly to 35 ºC, showed inhibitory effects on pollen germination of almond cv. 'Mamaei'. At a concentration of 0.05 mM putrescine and spermidine and 0.005 and 0.025 mM spermine caused longer pollen tube growth than that of the control at 10 ºC, while higher concentrations tended to inhibit pollen tube growth. At 25 ºC, most of the treatments had an inhibitory effect on pollen tube growth except for 0.25 mM putrescine and 0.005 mM spermine, which slightly stimulated pollen tube growth (SORKHEH et al., 2011). Addition of the polyamines biosynthesis inhibitors MGBG reduced polyamines biosynthesis, which indirectly reflected that polyamines are important to pollen germination and pollen tube growth. Conversely, treatments of the pollen of almond with 2.5 mM spermidine and 0.25 mM spermine during the germination and pollen tube growth increased intracellular spermidine and spermine concentrations. It is similar to the studies showing a strong positive correlation between conifer embryo development or its morphological quality and a high spermidine concentration (MINOCHA and MINOCHA, 1995). Exogenous polyamines serve merely as a nitrogen source for the plants (BAGANI and SERAFINI-FRACASSINI, 1985). Also, polyamines can act as free radical scavengers and protect senescing membranes against lipid peroxidation. Another possible mechanism is that polyamines might be active, not by themselves, but through their catabolic pathways or through their interaction with ethylene biosynthesis (KUMAR et al., 1997).

Ovule normality in response to temperature stress

Factors like ovule normality and longevity in fruit crops plays a decisive role in the effective pollination period, fertilization as well as determining fruit set. In higher plants fertilisation takes place after a pollen tube reaches a receptive ovule. However, various abnormalities during ovule development have been described in fruit species and these can often limit the EPP, pollination and fruit set. These abnormalities include incomplete development of ovule structures in sour cherry (FURUKAWA and BUKOVAC, 1989), and abnormalities in ovule or embryo sac development during flowering in olive (RALLO et al., 1981), almond (PIMIENTA and POLITO, 1982) and litchi (STERN et al., 1996; IRENAEUS, 2012). Moreover, in normally developed ovules, a short ovule life span has been reported to be an important factor limiting the EPP (CEROVIC and RUZIC, 1992b). In 'Granada' peach high temperature conditions delayed the female gametophytes (embryo sac) and promoted anomalies in the formation of male gametophytes. These promoted low pollen viability and a lack of synchrony in fertilization, thereby generating low fruit set percentages and yield (NAVA et al., 2009). Detrimental effect was observed in ovule normality of litchi at high temperatures during floral development (STERN et al., 1996). At extreme higher temperature (30/22 ºC), ovule normality was affected in all the cultivars and flowers with normal ovule was recorded better at 20/15 ºC (IRENAEUS, 2012). It was observed that litchi ovules lack embryo sac and egg cells (STERN et al., 1996). While some of these abnormalities appear to have a genetic basis, early degeneration of the ovule

seems to be environmentally affected and, thus, variable results can be obtained depending on location and year. The ovule degeneration is encompassed by callose layering at the chalazal end of the nucellus (STOSSER and ANSARI, 1982). Later it was shown that as callose accumulates at the chalaza, translocation to the ovule is interrupted (PIMIENTA and POLITO, 1982) and that this interruption is preceded by starch reduction in the ovule (RORIGO an HERRERO, 1998).

A shortening of style length, and abnormalities in ovary development have been reported under mild increases in temperature (an average of 3 ºC higher than control) during the last week of flower development in apricot (RORIGO an HERRERO, 2002), as well as under lower than optimal temperatures in mango (20/10 ºC, day/night) (SUKHBIVUL et al., 1999) resulting in lower fruit set. In 'Tainong 1' mango, proportion of deformed ovaries with a short style for samples collected when temperatures are low during floral development is greater than that for samples collected at "normal" temperatures, however there is no significant difference occurred (HUANG et al., 2010). The length of stigmatic receptivity is shortened at high temperatures and enlarged at low temperatures in sweet cherry and peach regardless of the effect on the male side (HEDHLY et al., 2003; HEDHLY et al., 2004). Temperature stress might affect stigma function by affecting the amount of exudates and their temporal availability to pollen grains (SRINIVASAN et al., 1999). Likewise, the analysis of carbohydrate content in chickpea flowers subjected to cold stress revealed a reduction in carbohydrate levels in aborted styles and ovaries compared to those retained in the plant (NAYYAR et al., 2005). A disruption in cell wall invertase activity was found under low temperature stress not only during the commonly reported pollen developmental phase but also in the stigma and the style (JAIN et al., 2007). Taken together, these findings point out to a plausible effect of temperature stress on the sporophytic structures of the pistil and warrant further research. Increasing the temperature from 5 ºC to 25 ºC in sweet and sour cherry (*Prunus cerasus* L.) reduced ovule longevity from 5 days to 1-2 days (POSTWEILER et al., 1985). Similarly, a constant temperature of 20 ºC in controlled growth chamber reduced ovule longevity in plum (*Prunus domestica* L.) when compared to both field conditions and a lower constant temperature (CEROVIC et al., 2000). A temperature above 25 ºC induced degeneration and/or suppression of embryo sac development in peach (KOZAI et al., 2004). Callose deposition at the ovule chalazal end has been traditionally used as a microscopic feature to assess early ovule degeneration (VISHNYAKOVA, 1991).

Genotypic variation in pollen characters and ovule normality in response to temperature stress

Temperature effects on pollen grain germination seem to be cultivar or species dependent and optimum temperature for pollen germination and tube growth depends on species and varies between cultivars (MERT, 2009; LOUPASSAKI et al., 1997). For example, in litchi, 'Floridian' is much more susceptible to high temperature than 'Mauritius', particularly during floral development but also with respect to Male1 (M_1) pollen germination *in vitro* at 35 ºC. 'Floridian' M_1 pollen germinated at 15 ºC, whereas, 'Mauritius' M_1 pollen did not (STERN and GAZIT, 1998). High temperature regime also had a more severe detrimental effect on gynocium development in 'Floridian' than 'Mauritius' (STERN et al., 1996). The difference between the two cultivars in their response to temperature may reflect the climate in their place of origin. 'Floridian' is related to 'Brewster' (DEGANI et al., 1995), which probably is identical to the known 'Fujian' cultivar 'ChenZee' (COBIN, 1954; GROFF, 1948). 'ChenZee' is cultivated mainly in Putian (25ºN latitude), whereas, 'Mauritius', which is apparently identical to the Chinese cultivar 'Tai So', is cultivated mainly in the southern parts of Fujian and Guangdong (22-23ºN latitude) (GAZIT and GOREN, 1997). Similarly, pollen performance in terms of viability, germination and tube growth were better from pollens of male2 (M_2) than that of the respective male1 (M_1) flowers in all the cultivars studied across different temperatures. In M_1 pollens, lowest pollen viability was observed at 30/22 ºC in all the cultivars and highest at 20/15 ºC while in M_2 flowers, the pollen viability was better at 20/15 ºC for cultivars China, Elaichi, Early Muzaffarpur and Rose Scented (93.89, 95.80, 96.41 and 92.60 %, respectively) and the viability decreased with increasing temperatures except for cultivar Nafarpal whose pollen viability was found the same at both 30/22 ºC and 26/20 °C (IRENAEUS, 2012). Significant differences were observed in the pollen quality (viability, longevity, morphological homogeneity, pollen germination and pollen tube growth rate) among six species and cultivars of each Prunus species including *P. dulcis, P. armeniaca, P. domestica, P. ceracus, P. salicina* and *P. avium* (SHARAFI, 2011). In sweet cherries, 20 °C was the optimum temperature for the *in vitro* germination of 'Bigarreu Gaucher' and 'Noble' while 25 °C was optimum for 'Starks' 'Bing' 'Gold' 'Vista', 'Van', '0900 Ziraat' and 'Stella' (KOYUNCU and GUCLU, 2009). Preincubation at 30 ºC decreased pollen germination for olive cutivars 'Koroneki', 'Kalamata' and 'Amigdalolia' but not for 'Mastoidis', confirming the strong genotype-treatment interaction observed in olive (FERNANDEZ-ESCOBAR et al., 1983). Pollen tube growth on the pistil and stigma receptivity suggested that different peach genotypes responded differently to different temperature treatment and selections Conserva 1566 and Conserva 693 and cv. 'Maciel' were tolerant to temperatures around 29 °C at beginning of blooming. Such differences in pollen germination and tube growth within the same species were also found in pear (CHAGAS et al., 2009; CHAGAS et al., 2010). In Juglans species, at 14 to 15 °C only pollen from early blooming varieties (Serr, Manregian, and Early Ehrhardt) germinated with 16.4 %, 2.2 % and 2.1 %, respectively. Pollens of the later-blooming *J. regia* cultivars, 'Idaho', 'Chico', 'Sharkey', 'Amigo', 'S. Franquetta', and 'Meylan' did not germinate at temperatures below 16 to 18 °C and germination percentages ranging from 1.4 to 6.2 % in that temperature range (LUZA et al., 1987). Best pollen germination for cultivars 'Şebin', 'Kaplan 86', 'Yalova 3', 'Pedro', 'Hartley' and 'Franquette' was noted at 27±1 °C (MERT, 2009) while for cv. 'Yunxin' germination occurred best at 25 °C (WU et al., 2008). Percent germination of pollen of 'Manchurian' crabapples and 'Golden Delicious' apple flowers on the stigmatic surface of 'Golden Delicious' pistils increased with increasing temperature from 13 to 29 °C in the first 24 and 48 h after pollination, respectively, but not thereafter. 'Manchurian' was a more effective pollinizer of 'Golden Delicious' than was 'Golden Delicious' pollen. For example, at 24 or 29 °C, some 'Manchurian' pollen tubes grew to the base of 'Golden Delicious' styles by 24 h after pollination. On the other hand, no 'Golden Delicious' pollen tube grew to the base of a 'Golden Delicious' style regardless of temperature and time (YODER et al., 2009).

Difference in pollen viability among the cultivars of hazelnut (HOSEINAVA et al., 2010) and germination of *Pistachio vera* L. (KAMIAB et al., 2007) was also obtained and pollen tube length ranged from 697 to 1270 μm (ACAR and KAKANI, 2010). In citrus, pollen performance is not only an inherent characteristic of the pollen genotype, but is largely dependent on the particular male-female combination and on genotype-temperature interactions (DISTEFANO et al., 2012).

Temperature requirement for the pollen germination and pollen tube growth in fruit species and cultivars may differ in early or late flowering period. For example, the desired temperature for optimum pollen germination of walnut was found comparatively low in early flowering cultivars than late flowering walnut counterparts (LUZA et al., 1987). A somewhat similar pattern of temperature responses by pollen was noted in a comparison of almond (*Prunus dulcis*) and peach (*P. persica*), two closely related species that differ in bloom

time (Weinbaun et al., 1984). Pollens from the earlier blooming almond had higher germination percentages at low temperatures and a lower temperature for maximum germination was needed. *In vitro* pollen germination and pollen tube growth of different Pistacia genotypes varied with temperature. The maximum percentage of pollen germination and pollen tube length of genotypes, and T_{min} and T_{max} were the most important parameters describing genotypic tolerance to low and high temperatures (Acar and Kakani, 2010). A heat shock of 40 °C is sufficient to induce mRNAs for heat shock proteins (HSP) in pollen of maize (Hopf et al., 1982) revealing the induced resistence of plant in order to protect (defence mechanism) the cells against damage and/ or death. High temperature proved to be lethal for the pollen of the cultivars studied and the determination of HSP mRNAs presence in the pollen might help explain why differential thermotolerance was observed among the cultivars studied.

Variations due to flower type in response to temperature stress

In crop like litchi, there are two type of male flowers; Male (M_1) flower and the pseudohermaphrodite (M_2) flower (functionally male) and differences in the pollen quality of this flower types has been reported. A consistent and usually significant advantage of M_2 over M_1 pollen was found when pollen from five cultivars (Mauritius, Floridian, No Mai Chee, Wai Chee and Early Large Red) was germinated *in vitro* under five different (15, 20, 25, 30 and 35 °C) temperature regimes (Stern and Gazit, 1998). The germination rate of M_2 pollen was consistently greater (P=0.01) than that of M_1 pollen in 'Wai Chee', 'No Mai Chee', and 'Early Large Red'. The maximal germination reached 55 % to 59 % for M_2 pollen, but only 8 % to 19 % for M_1 pollen. The optimal temperature for M_2 pollen germination was 30 °C in all cases. The same temperature was also optimal for the germination of 'No Mai Chee' M_1 pollen, whereas, germination of 'Wai Chee' and 'Early Large Red' M_1 pollen was greatest at 25 °C. Similarly, germination was better for M_1 pollens at 24 ºC in most of the cultivars and germination percentage decreased with increasing temperatures though for cultivar Bombai it was higher at 28 ºC. The pollens of M_2 flower in most of the cultivars showed better germination at 28 ºC though cultivars like Bedana, Elaichi and Nafarpal germinated better at 30 ºC. However, decrease in pollen germination was observed at lower and higher temperature extremes of 24 ºC and 32 ºC in all the cultivars (Irenaeus, 2012).

Earlier, higher germination rates for M_2 pollen were reported (Mustard et al., 1953; Costes, 1988) and such similarities in six cultivars (Fivaz et al., 1994) though no such advantage of M_2 over M_1 was recorded in cv. Bengal. Pollen tube growth rate was also reported to be faster for M_2 than M_1 pollen (Stern and Gazit, 1998; Irenaeus, 2012) with the maximum growth in cv. Rose Scented (Irenaeus, 2012). Higher fruit set was also obtained from the flowers pollinated by M_2 pollens. They opined that the difference in viability of the two pollens must be phenotypic as they were identical genetically. No difference in the size and shape of the pollens were found by (Stern and Gazit, 1998). It was thus, assumed that M_2 flowers secrete more nectar and sugar than M_1 flowers (Stern and Gazit, 1996) which indicates that the M_2 flower is a much stronger sink, and this may also manifest itself in a better supply of nutrients to its developing pollen.

Other factors affecting *in vitro* pollen germination and tube growth

Nutrient Media

Validity of the *in vitro* evaluation of pollen germination is a predictor of *in vivo* behaviour (Hormoza and Herrero, 1999; Tunistra and Wedel, 2000) and therefore it is important to standardize the nutrient media to get the best pollen germination and tube growth. And since the fruit set and yield largely depends on the pollen quality, nutritional requirements for successful pollen germination and pollen tube growth *in vitro* will help growers and researchers to supply the plant with different type of nutrients at proportionate amount. Correlation of pollen germination and ovule normality with nutritional requirements *in vitro* and *in vivo* leaf nutrient content at flowering may be considered for standardization of critical nutrient content. Also correlation studies of these parameters at different temperature regimes is important so as to categorize the genotypes for region specific recommendation and differential response of the genotypes to temperature may be due to variation in its metabolic process at different temperature. This response to nutrient media was found to vary from species to species and cultivar to cultivar.

Suitable culture medium for pollen germination *in vitro* of wild apricot was reported as No. D contained 1 % agar, 10 % sucrose, and 0.01 % boric acid, where pollen germination rates was 75.4 % and suitable concentration of sucrose and boric acid had certain promotion function for germination (Qiang et al., 2012). In avocado, maximum pollen germination could be achieved by using a medium containing 10 % sucrose and 23 % polyethylene glycol (PEG) (Alcaraz et al., 2011). The addition of PEG to the pollen germination medium was recommended in *Anacardium* (Subbaiah, 1984). Sucrose, boric acid, and calcium nitrate have been shown to be the key substrates for pollen germination in other species (Tunistra and Wedel, 2000). Similarly use of sucrose and agar supplemented with boron as culture medium for pollen germination and tube growth has been done in Surinam cherry (*Eugenia uniflora* L.) (Franzon et al., 2007). When the concentration of sucrose was 30 g l^{-1} and boric acid was 3 g l^{-1}, the internal and external osmotic pressure of cherry cv. 'Duan bing' could keep balance and the ratio of pollen germination was the highest (Qiang et al., 2009). 15 % sucrose concentration gave the highest germination rates for walnut cultivars (Sutyamez, 2007). Other researchers also noticed that, 15 % and 20 % sucrose concentration gave the highest germination rates in various fruit species (Sutyamez and Eti, 2006; Asma, 2008). Generally, the best pollen germination rates in most of the walnut cultivars studied were obtained from 15 % and 20 % sucrose concentrations while in cv. 'Pedro' best germination rates were obtained with 10 % sucrose concentration (Mert, 2009). However, 100 g sucrose+10 mg boric acid+40 mg $CaCl_2$ l^{-1} was found best for germination of cv. 'Yunxin' (Wu et al., 2008).

In pear, use of 10 g l^{-1} of agar combined with 90 g l^{-1} sucrose for the rootstock 'Taiwan Mamenashi' and 47.78 g l^{-1} for the 'Taiwan Nashi-C', with 795 to 838 mg l^{-1} of boric acid, under absence of calcium nitrate and pH between 5.2 and 5.8 and temperature of 28 °C, provided the best conditions to the germination of the pollen grains (Chagas et al., 2010). Similarly, for the Aurora 1 cultivar, higher germination of pollen grains was obtained with the use of 48.29 g l^{-1} of sucrose, 10 g l^{-1} of agar, 400 mg l^{-1} of boric acid and pH 5.5. For the Douradao cultivar, higher germination was obtained on medium containing 90 g l^{-1} of sucrose, 10 g l^{-1} of agar, 400 mg l^{-1} of boric acid, 369 mg l^{-1} of calcium nitrate and pH 6.5 (Chagas et al., 2009). However, optimum temperature for germination was recorded to be 25 °C in both the cultivars. Sucrose plays a nutritive role for pollen germination and no germination occurred in avocado at a concentration less than 5 % sucrose. Variations in the effect of different sucrose concentrations are associated to different osmotic potentials (Alcaraz et al., 2011). Sucrose acted in the regulation of osmotic optimal conditions and nutritional source for pollen germination and pollen tube development (Taylor and Heper, 1997). PEG is an osmotic regulator not metabolized in pollen that is thought to regulate the permeability of the plasma membrane (Shivanna and Sahwney, 1995) and can be highly effective to promote pollen germination and reduce bursting (Vasil, 1987), although its mechanism of action is not well understood.

Duration of incubation for germination and growth

Pollen grains showed different rates of viability and germinability in relation to storage length, storage temperature, genotype and their interactions in four olive cultivars viz., 'Carolea', 'Frantoio', 'Leccino' and 'Moraiolo' though no linear correlation has been found between viability and germination rates (FERRI et al., 2008). The maximum percentage of germinated pollen is obtained with eight hours after inoculation for 'Taiwan Nashi-C' and twelve hours for 'Taiwan Mamenashi' pear (CHAGAS et al., 2010).

Poor fruit set due to temperature stress

Many cultivated and wild plant species within different climatic regions worldwide perform poorly and display erratic fruit and seed set when subjected during their flowering phase to high and low temperatures beyond their optimum needs. For example, in temperate fruit trees, a substantial reduction in fruit set has been shown in apricot (*Prunus armeniaca* L.) after an increase in the average daily temperature as low as 3 °C during the week preceding anthesis (RODRIGO and HERRERO, 2002), or in sweet cherry (*Prunus avium* L.) during the first two weeks following anthesis (HEDHLY et al., 2007). Similarly, results in peach (*Prunus persica* L. Batsch), show that a two month exposure to different constant temperatures in growth chambers, starting one month before anthesis, resulted in very low fruit set at above 25 °C (KOZAI et al., 2004). Cherimoya (*Annona cherimola* Mill.), native to the tropical highlands of the Andes, usually displays lower fruit set in tropical lowlands and in temperate regions. An increase in temperature from 20 °C to 30 °C during two consecutive years substantially reduced fruit set and increased the number of malformed fruits (HIGUCHI et al., 136).

Mango has been shown to be prone to recurrently erratic fruit set with an increased proportion of stenocarpic fruits when temperature falls below 10 °C during flowering (SUKHVIBUL, 2005). Low temperatures adversely affected inflorescence and floral development (ISSARAKRAISILA et al., 1992), causing morphological changes in styles, stigmas, ovaries and anther size in 'Nam Dok Mai', 'Kensington', 'Irwin' and 'Sensation' cultivars (SUKHVIBUL et al., 1999). Sternospermocarpic mangoes were reported to be caused by low temperatures (RAM et al., 1976). Low temperature during flowering might also interfere fertilization and/or ovule development in early stage of fruit development and hence resulted in sternospermocarpic fruit set (LIM et al., 1996). A tendency towards ovule degeneration, both in pre-fertilised and post-fertilised stages may seriously impair fruit set. This appears to be a specific genetic character in 'Doyenne du Comice' pear that is responsible for the poor fruit set of this cultivar (JAUMIEN, 1968). In the same way, the high proportion of both degenerated embryo sacs and embryo sacs that lack a functional egg cell is responsible for the poor fruit set of 'Constant' apricot, regardless of weather conditions (EATON and JAMONT, 1965).

Conclusion

A wide variation exists not only among the species in terms of pollen viability, germination, pollen tube growth, ovule normality and the specific requirements for the reproductive process to occur but also among the cultivars within the same species. Also variation in these characters are not only genotypic but is also influenced greatly by temperature. Optimum temperature at which such reproductive process can best occur has been assessed and established in many crop species and cultivars. Nutritional requirements for *in vitro* germination and growth of pollens has also been standardized for many crop species and this can be considered for establishing *in vivo* nutritional requirements and standardizing critical leaf nutrient standards. Accordingly, cultivars with higher pollen viability and germination and or higher temperature stress tolerance can be identified and selected for use as pollinizers, breeding purpose and region specific adoption based on temperature prevalence in the area. Pollen release and germination ability might be suggested as a good criterion for determining plant response to high temperature and this can be used as selection criteria in breeding programmes for selecting heat tolerant genotypes. Poor fruit set and yield may be largely attributed to impairment of pollen and ovule quality under high temperature is the most important factors to determine the fruit set ability. Pollen performance is also dependent on the particular male-female combination and on genotype-temperature interactions as in citrus (DISTEFANO et al., 2012).

Decreased pollen number and viability at high temperature may be due to anther indehiscence, polyamine synthesis inhibition, degeneration of tapetum layer and/or decreased carbohydrate metabolism, while, there are contrary reports that at higher temperature, impairment of pollens is due to higher sucrose and starch accumulation as a result of reduced utilization and starch degradation rather than biosynthesis prior to anthesis. Though the mechanisms in the reduction of pollen quality under low temperature has also been attributed to alterations in starch accumulations and decreased respiratory activity, the precise nature leading to poor pollen development is still not clear. Depolymerization of actin cytoskeleton in low temperature stress is also reported which induce activation of pollen plasma membrane that prevents pollen tube growth (WU et al., 2012). It may also be concluded that embryo sac degeneration, shortening of stigma receptivity, reduced carbohydrate availability and style abortion occurs under temperature stress which are important factors to determine the fruit set ability.

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