TEMPERATURE SENSITIVITY OF FOOD LEGUMES: A PHYSIOLOGICAL INSIGHT

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ABSTRACT

Of the various environmental stresses that a plant can experience, temperature has the widest and most far-reaching effects on legumes. Temperature extremes, both high (heat stress) and low (cold stress), are injurious to plants at all stages of development, resulting in severe loss of productivity. In response to unfavorable temperatures, plant biomolecules such as stress proteins, enzymatic and non-enzymatic antioxidants, organic osmolytes and phytohormones come into play. Usually, their endogenous levels go up as a part of the plant's defense mechanism. The expressions of these molecules, which may be useful as metabolic indicators of stress tolerance, depend on the plant species exposed to the stress and the intensity and duration of the temperature stress. Some of these molecules such as osmolytes, antioxidants (non-enzymatic) and phytohormones may be supplied exogenously to improve temperature stress tolerance. Legumes, especially food legumes such as chickpea, lentil, mungbean, soybean and peas, show varying degrees of sensitivity to high and low-temperature stresses which reduces their potential performance at different developmental stages such as germination, seedling emergence, vegetative phase, flowering and pod/seed filling phase. The reproductive stage is considered highly sensitive to abnormal temperatures, and both cold and high-temperature stresses can impair the development and function of male and female components leading to loss of flowers and pods. Additionally, pod filling is hindered due to inhibitory effects of unfavorable temperatures on enzymatic processes linked to sucrose synthesis and its transport to developing sinks (seeds). To address the ever-fluctuating temperature extremes that various legumes are being constantly exposed, efforts are being made to develop tolerant plant varieties via

conventional breeding methods and, more recently, using molecular breeding techniques. Here, we review the progress made towards the adverse effects of abnormal temperatures on growth and physiology of food legumes and propose appropriate strategies to resolve these effects.

INTRODUCTION

Temperature is one of the most important factors determining where crops are grown and depends on their temperature sensitivities (Repo et al. 2008) that affect their phenology and yield (Hayashi 2001). Crops are exposed to a wide range of temperature fluctuations under natural conditions during growth. Temperature instabilities may be experienced by crops at micro or macro-environment levels, but both can have serious implications on normal growth and production. Climatic hazards are likely to increase in the near future and plants will face lethal temperature extremes (Solomon 2007) leading to a pragmatic shift in temperature zones, differential rainfall patterns and agricultural production belts (Justus and Fletcher 2006). Considering this, several studies have evaluated different plant species for their responses to temperature stress, e.g. tomato (*Solanum lycopersicum* ; Sato et al. 2000), beans (*Vicia faba*; Hamada 2001), mustard (*Brassica* spp.; Morrison and Stewart 2002), cotton (*Gossypium* spp.; Brown et al. 2008), *Arabidopsis thaliana* (Deng et al. 2011), soybean (*Glycine max*; Board and Kahlon 2011), chickpea (*Cicer arietinum*; Kaushal et al. 2013) and tobacco (*Nicotiana tabacum*; Cui et al. 2013). Various cool-season legumes (chickpea, lentil, pea, faba beans) are susceptible to rising temperatures, as indicated by experiments in the field and a controlled environment (Stoddard et al. 2006). Each legume species has its own range of maximum and minimum temperatures, termed its threshold temperature (Table 1), and extreme variations in temperature, both high and low, will have serious repercussions on every stage of plant development (Zinn et al. 2010), resulting in severe loss of productivity.

Table 1: Threshold temperature range of some legumes.

LEGUMES	THRESHOLD TEMPERATURE RANGE (°C)	REFERENCES
Pea *(Pisum sativum)*	15–20°C 20–21°C	Mahoney 1991 Fletcher et al. 1966
Lentil (*Lens culinaris*)	15–25°C	Barghi et al. 2012 Roy et al. 2012
Chickpea (*Cicer arietinum*)	15–30°C	Singh and Dhaliwal 1972 Wang et al. 2006
Cowpea (*Vigna unguiculata*)	18–28°C	Craufurd et al. 1997
Pigeon pea (*Cajanus cajan*)	18–29°C	Duke, 1983
Common beans *(Phaseolus vulgaris)*	20–24°C	Kigel et al. 1991 Konsens et al. 1991
Soybean (*Glycine max*)	26–36°C	Boote et al. 2005 Hatfield et al. 2008
Mung bean *(Vigna radiata)*	28–30°C 30–40°C	Poehlman, 1991 Tickoo et al. 1996
Groundnut (*Arachis hypogea*)	30–35°C	Talwar et al. 1999 Prasad 2000

Globally, legume production ranks third preceded by cereals and oilseeds (Popelka et al. 2004), contributing up to ~27% of total crop production (Graham and Vance 2003). The principal grain legumes in order of their respective global consumption are common beans, pea (*Pisum sativum* L.), chickpea, broad bean (*Vicia faba* L.), pigeon pea (*Cajanus cajan* L.), cowpea (*Vigna unguiculata* L.) and lentil (*Lens esculentum* L.; Reddy et al. 2012). Legumes, especially grain legumes, account for about 33% of dietary protein requirements, serve as an animal feed and fodder, and a source of income for small farmers, especially in developing and under-developing countries (Vance et al. 2000; Popelka et al. 2004). These are ecologically desirable but despite these facts, pulses are regarded as secondary crops worldwide, with the global area under cereal cultivation about ten times higher than that of pulses (Akibode and Maredia 2011). The human population will grow to around 9 billion by 2050 and the demand for world food production is expected to rise by 70%. It will be challenging for agricultural experts to meet the food demand of the growing population, e.g. global legume demand has increased from 26–27 million tons in

1975–76 to 43–44 million tons in 2007 (FAOSTAT; http://faostat3.fao.org; Fig. 1) while production has not increased accordingly (Fig. 2).

Several abiotic and biotic factors limit the production potential of legumes (Jahansen et al. 1994; Dita et al. 2006) with temperature stress as one of the most important (Gaur et al. 2008, 2011; Kumar et al. 2010). Global climate change is resulting in extreme temperature (high or low) situations in different regions of the world (Wheeler et al. 2000; Porter and Semenov 2005) which affects the performance of winter- as well as summer-season legumes (Stoddard et al. 2006; Board and Kahlon 2011). It is important to understand the responses of various legumes to high or low stressful temperatures in order to address their stress tolerance to ensure global food availability, now and in the future.

In this background, the present review provides information on the responses of various important legumes to low and high-temperature regimes at different organizational levels and proposes suitable measures to manage such temperature stresses.

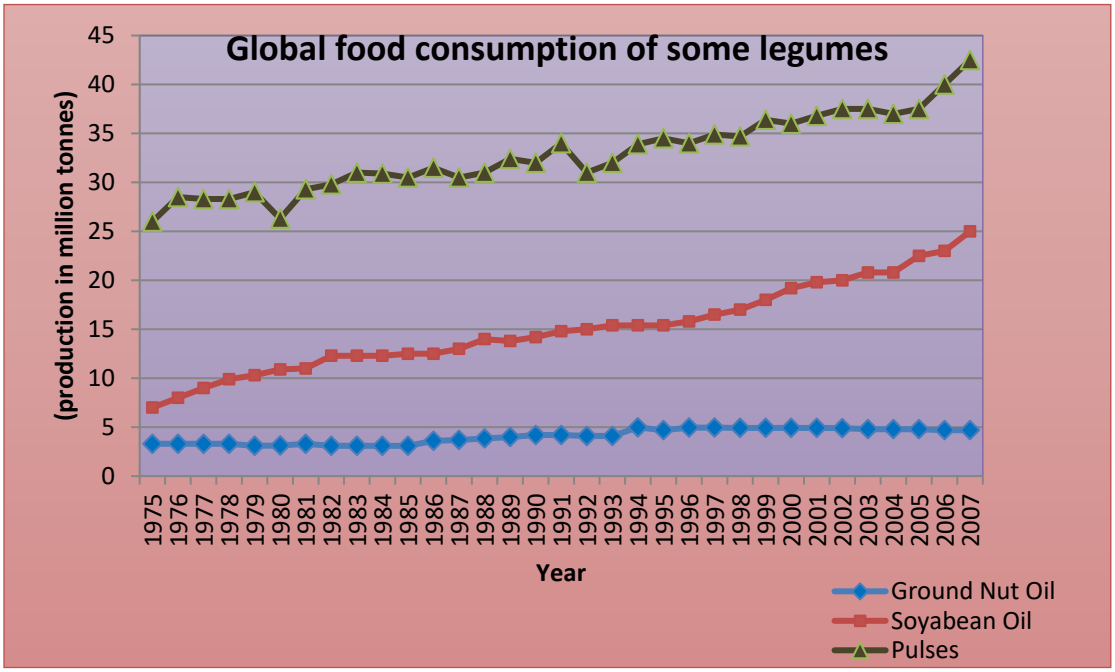

Fig. 1: Global food consumption of some important legumes from 1975 to 2007. Pulses and soybean oil demand increased while that of groundnut oil hardly changed. Source: FAOSTAT.

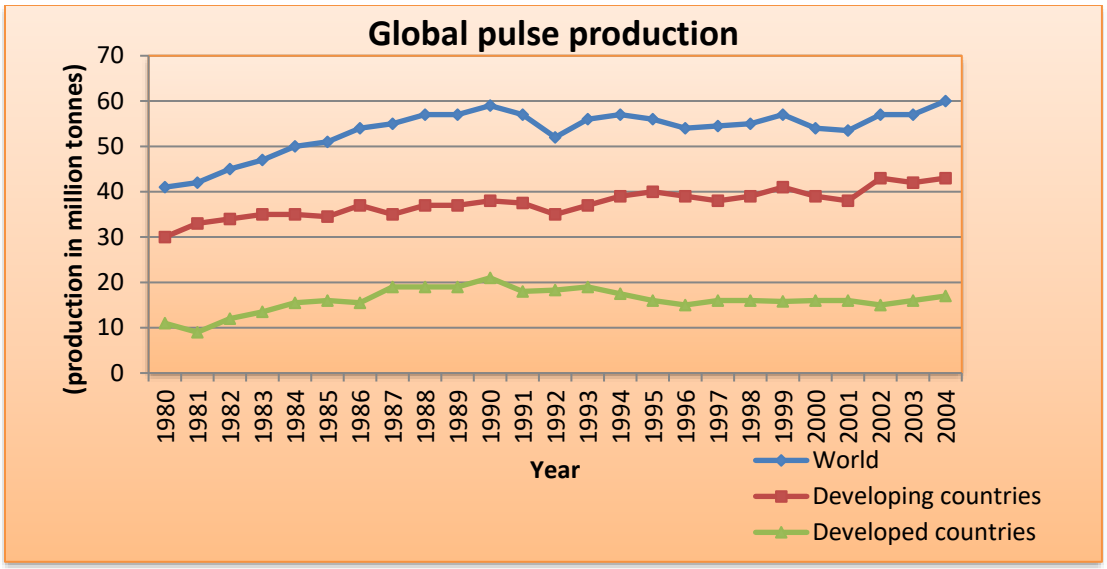

Fig. 2: Global pulse production from 1980 to 2004. Source: FAOSTAT.

Prolonged exposures to stressful temperatures are bound to impair plant growth and function. The effects of such are summarized below in the context of legumes.

1. VEGETATIVE PHASE

The first stage of plant development, i.e. seed germination, is prone to temperature stress; each plant species has an optimum temperature for maximum germination. Unfavorable temperatures can directly affect seed germination and seedling emergence rate, and early survival and growth of seedlings, e.g. in chickpea, long periods of chilling during germination enhance its susceptibility to soil-borne diseases, and poor crop establishment adversely affects seedling growth and may even result in the death of young plants (Croser et al. 2003). Chickpea plants are chilling sensitive and the effects of low temperature have been studied in the field and under controlled conditions (Kaur et al. 2008; Heidarvand et al. 2011). These studies confirmed that chilling-sensitive genotypes had lower survival percentages at temperatures less than 10°C. One day of chilling shock (5°C) in faba bean (broad beans) impaired plant growth (Hamada 2001). Similarly, low temperatures (1°C) induced damage in the early vegetative phase of soybean (Posmyk et al. 2005) and peas (Badaruddin and Meyer 2001). In some cases, complete seedling death has been reported (Meyer and Badaruddin 2001). Frost damage also exposes peas to various fungal diseases which intensifies the damage (Knott and Belcher 1998).

High temperatures are also potentially harmful to seed vigor, seed germination, seedling emergence and survival (Wahid et al. 2007). High temperature-induced inhibition of seed germination has been observed in various legumes, e.g. soybean, pea, bean (Nemeskèri 2004), alfalfa (*Medicago sativa*; Mingpeng et al. 2010), mungbean (*Vigna radiata*; Kumar et al. 2011a), and chickpea (Kaushal et al. 2011; Piramila et al. 2012). Growth of pea seedlings declined, when subjected to heat shock (45°C, 50°C) but surprisingly, the effects of heat injury were circumvented by exposing the heat-stressed seedlings to chilling temperatures (Shereena and Salim 2006). Heat tolerance in three prominent legumes- beans, pea and soybean (Nemeskèri 2004) was investigated under different temperature regimes-control (20°C/10°C as day/night temperature), moderate high stress (MHT; 25°C/25°C as day/night temperature) and serious heat stress (SHT; 30°C/30°C; day/night). In soybean and beans, SHT treatment for 8 days resulted in 50.4 and 36.2% dead seeds respectively, under non-irrigated conditions whereas, under irrigated conditions the respective values reached 87.6 and 36.8%. Root lengths of 4 and 8 days old seedlings were also measured in bean, pea and soybean. The greatest reduction was recorded under SHT (30°C/30°C as day/night temperature) condition and the pea seedlings were worst affected. The root lengths of 8 days old heat-stressed seedlings of bean, pea and soybean drastically decreased to 5.3, 3.04 and 3.10 cm as compared to their respective controls in which root lengths recorded were 16.96, 15.04 and 16.56, respectively. Likewise, severe damage to mungbean seedlings was noticed when subjected to 50°C for 2 h, as evident from decreased mean seedling length and heat tolerance index (Mansoor and Naqvi 2011). Alfalfa plants are also adversely affected by high temperatures resulting in stunted growth, increased susceptibility to diseases and even plant death in extreme cases (Mingpeng et al. 2010). Similarly, heat-stress (35–40°C) experiments on lentils reduced germination and retarded seedling growth (Chakraborty and Pradhan 2011). A comparative evaluation of all legumes under a similar set of experimental conditions would provide better insight into their relative sensitivities to low and high temperatures.

2. REPRODUCTIVE DEVELOPMENT

The reproductive stage is the most susceptible to temperature stress (Hedhly et al. 2008; Thakur et al. 2010). Unfavorable temperatures at this stage inhibit flower set and flower retention, impair normal development of male and female gametophytes, followed by ovule abortion, reduced fruit set and impaired grain filling (Fig. 3), eventually leading to yield losses (Table 2). Cool-season

legumes, i.e. chickpea, lentil, pea and faba beans are sensitive to low temperature, especially during pod formation and seed filling (Maqbool et al. 2010). Low temperatures can impair carbohydrate metabolism resulting in energy-deprivation of various reproductive tissues such as tapetum, style and endosperm causing infertile male and female gametophytes (Nayyar et al. 2005b; Oliver et al. 2005), e.g. exposing chickpea to temperatures <15°C during the reproductive stage significantly impaired flower, pollen and pod set, and thus reduced yield (Berger et al. 2005, 2006; Nayyar et al. 2005c; Kumar et al. 2010). Chickpea plants exposed to cold stress often produce distorted anthers with sterile pollen grains resulting in reduced fertilization (Nayyar et al. 2005b, Thakur et al. 2010). Failed fertilization has been attributed to impaired pollen tube growth in the style thus leading to no pod set in cold-stressed chickpea (Clarke and Siddique 2004). Observations in chickpea were further corroborated by fluorescence studies (Kumar et al. 2010) which identified the loss in pollen load, reduced pollen germination on the stigmatic surface and reduced pollen tube growth under very low temperatures, thus leading to fertilization failure.

Grain filling depends on the source–sink relationship which declines under low temperature due to a reduction in the duration and rate of grain filling and inhibition of accumulation of storage proteins, minerals and amino acids, as reported in chilling-stressed chickpea plants (Nayyar et al. 2007). Low-temperature damage to phenology and grain filling in legumes has been well documented in pea (Guilioni et al. 1997), chickpea (Nayyar et al. 2005c; Berger et al. 2006), soybean (Kokubun et al. 2001; Ohnishi et al. 2010) and pigeon pea (Sandhu et al. 2007). The chilling injury inflicted on soybean (Funatsuki et al. 2003, 2004; Kurosaki and Yumoto 2003) revealed that at temperatures <15°C irreversible yield loss occurred (Gass et al. 1996). Cold exposure to two soybean varieties—Hayahikari (cold tolerant) and Toyomusume (cold-sensitive)—reduced yield and yield-related components, and was more pronounced in Toyomusume (Kurosaki and Yumoto 2003). Flowering and podding are the most cold-susceptible stages in soybean (Board and Kahlon 2011), exposure to cold stress at these stages reduced soybean yield by up to 70% compared with exposing the same stress at maturity. Severe chilling injury was observed in pea accessions from 34 countries at flowering and podding (Shafiq et al. 2012). The low-temperature damage in pea was manifested in the form of aborted buds, flowers and fruits (pods) and smaller seeds. Such observations could be used to screen for cold resistant and frost-tolerant accessions of pea and to develop new frost-tolerant varieties. .

Heat stress is a critical abiotic stress responsible for reduced yields and dry matter production in many crops worldwide (Giaveno and Ferrero 2003). A rise in temperature may initially accelerate plant growth (Gan et al. 2004) and reproductive development but eventually, it limits the development of various yield components (Hall 2004; Boote et al. 2005). A rise of 1–2°C above the threshold temperature is enough to impair yield in important leguminous crops such as cowpea (Hall 1992; Ahmed and Hall 1993), groundnut (Prasad et al. 1999), common bean (Rainey and Griffiths 2005), lentil (Barghi et al. 2012) and chickpea (Gaur et al. 2008; Devasirvatham et al. 2012; Kumar et al. 2013). Although, the relative heat sensitivity varies for different crops (Sung et al. 2003), it is proposed that an average 1°C increase will reduce plant yield by at least 3–4% (Mishra 2007). In a controlled environment, 40°C/30°C (day/night) significantly reduced yield in heat-sensitive chickpea genotypes compared with tolerant genotypes. A further increase to 45°C/35°C inhibited pod set completely (Kumar et al. 2013). High temperatures reduced yield and yield-related attributes such as dry matter accumulation and partitioning (Omae et al. 2007; Kumar et al. 2008a), pod set, pod dry weight and harvest index in snap beans (Omae and Kumar 2006; Kumar et al. 2008a).

The relationship between yield loss and heat stress was so strong that yield and related attributes were advocated in the screening of heat-tolerant and heat-sensitive chickpea genotypes (Krishnamurthy et al. 2011). The yield of bean crops raised during winter and summer differed with the winter-sown crop having 41 and 38% more biomass and yield, respectively, than the summer crop (Escalante-Estrada et al. 2001). In soybean, temperatures above 40°C resulted in reduced pod set, seed production and yield (Board and Kahlon 2011) which agrees with other heat-stress experiments in soybean (Kitano et al. 2006, Djanaguiraman et al. 2011). In snap bean (*Phaseolus vulgaris*), heavy yield losses under high temperatures were attributed to less water in floral parts and leaves due to an increase in transpiration (Tsukaguchi et al. 2003).

Green bean (*P. vulgaris)*, genotype ‘Haibushi’ (heat tolerant), had higher pollen viability than its heat sensitive counterpart ‘Kentucky Wonder’ (Suzuki et al. 2001) which also produced abnormal pods and abscised flowers at high temperatures (Suzuki et al. 2003). The higher sensitivity of Kentucky Wonder to high temperature was attributed to the greater loss of relative tissue water content (RWC) and leaf water potential (LWP; Omae et al. 2005), a finding that was further corroborated by Tsukaguchi and Egawa (2006). Ultrastructural studies revealed that pollen sterility was due to degenerated tapetum owing to heat stress (Suzuki et al. 2001). These

findings were confirmed by exposing hydroponically-grown beans to high temperature which resulted in an overall reduction in plant growth including poorly-developed roots, reduced leaf area and reduced dry weight (Incrocci et al. 2000). High temperature also affected pollen germination and pollen tube growth. In lentils, 15°C was the most favorable temperature for pollen germination and pollen tube growth, with higher temperatures adversely affecting pollen tube growth (Barghi et al. 2013). Further studies are needed to dissect the sensitivity of various reproductive stages to low and high temperature. In addition, molecular mechanisms of pollen development under cold or high-temperature stresses including genes targeted for temperature stresses need to be elucidated. Identifying mechanisms associated with reproductive temperature tolerance at different organizational levels in various legumes will be achieved more easily if contrasting genotypes with matching phenology are used.

Not only cool-season legumes are susceptible to high temperatures; some warm-season legumes such as cowpea have experienced a reduction in pod set in response to moderately-high night temperatures (Thiaw and Hall 2004). Thus, various legumes are sensitive to both low- and high-temperature stress from the vegetative to reproductive stage, resulting in metabolic and reproductive dysfunction and low yields (Table 3).

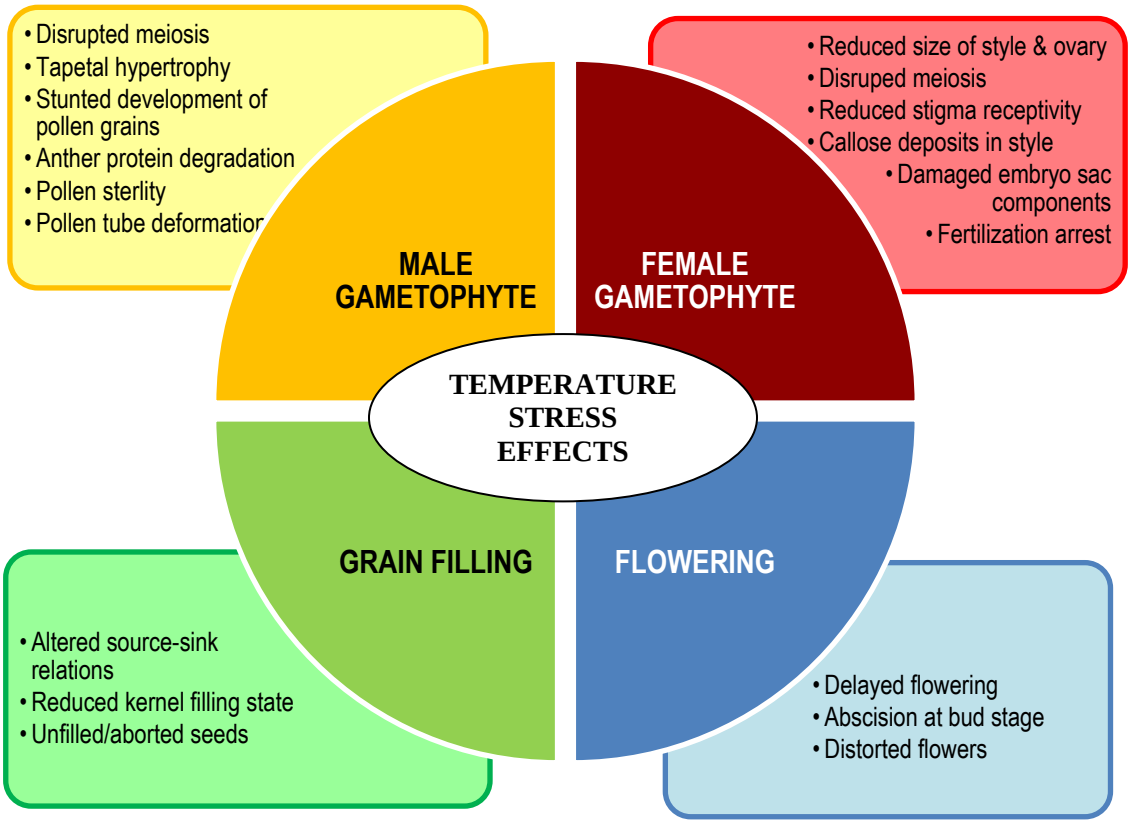

Fig. 3: Various effects of temperature stress on reproductive development stages. Temperature stress during the reproductive phase may result in delayed flowering, distorted flowers, impaired male and female gametophyte development followed by ovule abortion, reduced fruit set, impaired grain filling and eventually yield losses

Table 2: Temperature-sensitive reproductive developmental stages in various legumes. The effects are indicated along with respective references.

DEVELOPMENTAL STAGE	EFFECTS	COLD STRESS (References)	HEAT STRESS (References)
Pre-fertilization	Impaired microsporogenesis and megasporogenesis	*Cicer arietinum* (Kumar et al. 2010) *Glycine max* (Ohnishi et al. 2010)	*Phaseolus vulgaris* (Porch and Jahn 2001; Suzuki et al. 2001)
	Loss of pollen viability	*Cicer arietinum* (Kumar et al. 2010)	*Cicer arietinum* (Kumar et al. 2013)
	Loss of pollen germination	*Cicer arietinum* (Srinivasan et al. 1999) *Glycine max* (Koti et al. 2004; Salem et al. 2007)	*Phaseolus vulgaris* (Porch and John 2001) *Arachis hypogea* (Kakani et al. 2005) *Cicer arietinum* (Kumar et al. 2013) *Lens* spp. (Barghi et al. 2013)
	Pollen tube growth inhibition	*Cicer arietinum* (Clarke and Siddique 2004; Kumar et al. 2010) *Glycine max* (Koti et al. 2004; Salem et al. 2007)	*Arachis hypogea* (Kakani et al. 2005) *Cicer arietinum* (Kumar et al. 2013) *Lens* spp. (Barghi et al. 2013)
	Loss of stigma receptivity	*Cicer arietinum* (Nayyar et al. 2005b; Kumar et al. 2010)	*Cicer arietinum* (Kumar et al. 2013)
	Loss of ovule viability	*Cicer arietinum* (Srinivasan et al. 1999; Nayyar et al. 2005b)	*Cicer arietinum* (Jakobsen and Martens, 1994) *Phaseolus vulgaris*: (Gross and Kigel, 1994)
	Abscised flowers	*Pisum sativum* (Shafiq et al. 2012)	*Phaseolus vulgaris* (Suzuki et al. 2003) *Glycine max* (Board and Kahlon 2011)
Fertilization	Fertilization arrest	*Cicer arietinum* (Clarke and Siddique, 2004) *Glycine max* (Ohnishi et al. 2010)	*Cicer arietinum* (Kumar et al. 2013)
Post-fertilization	Reduced embryogenesis	*Cicer arietinum* (Srinivasan et al. 1999; Nayyar et al. 2005b)	*Cicer arietinum* (Kumar et al. 2013)
	Decreased ovule no. and increased ovule	*Cicer arietinum* (Srinivasan et al. 1999)	*Cicer arietinum* (Kumar et al. 2013)

	abortion		
	Abnormal pod formation and seed filling	*Glycine max* (Kurosaki and Yumoto, 2003; Funatsuki et al. 2004)	*Phaseolus vulgaris* (Suzuki et al. 2003)
	Poor seed set	*Cicer arietinum* (Berger 2005; Nayyar et al. 2005c; Kumar et al. 2010)	*Glycine max* (Board and Kahlon 2011)
		Pisum sativum (Shafiq et al. 2012)	*Cicer arietinum* (Kumar et al. 2013)

229
230 **Table 3: The proposed stressful temperature ranges for various legumes and their effects.**

PLANT	STRESSFUL TEMPERATURE	STAGE	EFFECT	REFERENCE
Glycine max	42–43°C	Vegetative stage	PSII damaged	Ferris et al. 1998
	Above 35°C	Reproductive stage	Flower abscission	Koti et al. 2004
			Reduced yield	Salem et al. 2007
Cicer arietinum	Below 15°C	Reproductive stage	Flower abscission	Srinivasan et al. 1999
			Reduced pod set	
		Grain filling	Reduced yield	Clarke and Siddique 2004 ; Berger et al. 2005, 2006 ;Nayyar et al. 2005c
	35/16°C	Flower and pod formation	Reduced yield	Gan et al. 2004
				Wang et al. 2006
	45/35°C	Reproductive stage	PS II damage	Kumar et al. 2013
	Above 32/20°C		Reduced RUBISCO activity and sucrose content	Kaushal et al. 2013
Vicia faba	42°C for 1 day	Vegetative stage	Impaired growth, decreased photosynthesis	Hamada 2001
	5°C for 1 day	Vegetative stage	Impaired growth, decreased photosynthesis	Hamada 2001
Phaseolus vulgaris	10°C	Vegetative stage	PS II damage	Tsonev et al. 2003
Cajanus cajan	Below 10°C	Seed germination till early growth	High mortality	Sandhu et al. 2007
Pisum sativum	–4.8°C for 4 h	Reproductive stage	Flower abscission	Shafiq et al. 2012
			Reduced pod set	
		Grain filling	Reduced yield	

3. CELL MEMBRANES

According to the lipid bilayer model of biomembranes (Singer and Nicholsan 1972), the cellular membrane consists of a phospholipid bilayer sandwiched between proteins. The membrane system is fluid-mosaic in nature, flexible, semi-permeable, exists in two forms i.e. liquid crystalline as well as solid gel-phase, and the inter-conversion is temperature dependent (Fig. 4). Thus, under low temperatures, membranes get more static by changing into the gel phase thereby reducing their fluidity and causing membrane rigidification resulting in loss of membrane function (Vigh et al. 2007; Jewell et al. 2010). The phase transitions in mungbean were acknowledged as a pioneering study on legumes (Raison and Orr 1986). Membrane damage can be measured using an electrolyte leakage test. Five-day-old seedlings subjected to a stressful low temperature (4°C) had an irreversible chilling injury from increased electrolyte leakage (Chang et al. 2001). The solute leakage apparently resulted from disruption of the plasma membrane and tonoplast but if roots were kept at an ambient temperature (28°C) while shoots remained exposed to 4°C, the chilling injury declined. Another study confirmed the chilling-induced electrolyte leakage and lipid peroxidation in mungbean (Saleh 2007). Chilling-inflicted membrane damage was also reported in broad bean (Hamada 2001) and chickpea (Croser et al. 2003; Nayyar et al. 2005a).

Membranes are also the primary sites of injury under heat stress (Blum 1988; Wise et al. 2004) since high temperature affects membrane structure and function (Weis and Berry 1988; Wahid et al. 2007) by increasing membrane fluidity (Liu and Huang 2000; Howarth 2005), and activates the lipid-based signaling cascade (Saidi et al. 2009; Ruelland and Zachowski 2010; Hovarth et al. 2012). Cellular membranes are susceptible to heat injury due to structural modification of component proteins leading to increased membrane permeability and hence increased electrolyte leakage. Thus, electrolyte leakage values serve as indicators of membrane damage and have been used to evaluate the thermostability of cell membranes under heat stress. The effects of high temperature on membranes have been studied in mungbean (Collins et al. 1995) and chickpea (Kumar et al. 2013). Higher membrane damage was observed in sensitive chickpea genotypes at 40/30°C, which was further aggravated at 45/35°C (Kumar et al. 2013). The extent of membrane injury can be thus used as a criterion for evaluating relative heat sensitivity of different genotypes as studied in soybean (Martineau et al. 1979), chickpea (Ibrahim 1994) and cowpea (Ismail and Hall 1999). Similarly, Srinivasan et al. (1996) tested cell membrane thermostability

and correlated it with heat sensitivity in various legumes in the following order: groundnut (most tolerant) > soybean > pigeon pea > chickpea (most sensitive). Likewise, Ibrahim et al. (2011) evaluated membrane thermostability in cool-season legumes (chickpea, faba bean and lentil) and found it closely-related to plant heat tolerance. Recently, the heat tolerance of nine lentil genotypes in terms of biomembrane stability was evaluated by subjecting them to different temperatures ranging from 15–45°C at 10°C intervals (Barghi et al. 2013). The findings proposed that 15°C is the most favorable temperature with further increases in temperature increasing electrolyte leakage to suggest that the heat stress inflicted membrane damage. Heat-induced membrane damage has also been reported in broad bean (Hamada 2001; Mansoor and Naqvi 2013), chickpea (Tangden et al. 2006; Kumar et al. 2013) and soybean (Djanaguiraman et al. 2011). Membrane damage can be considered a reliable indicator of stress tolerance in legumes and can be effectively employed to screen these crops for cold or heat tolerance.

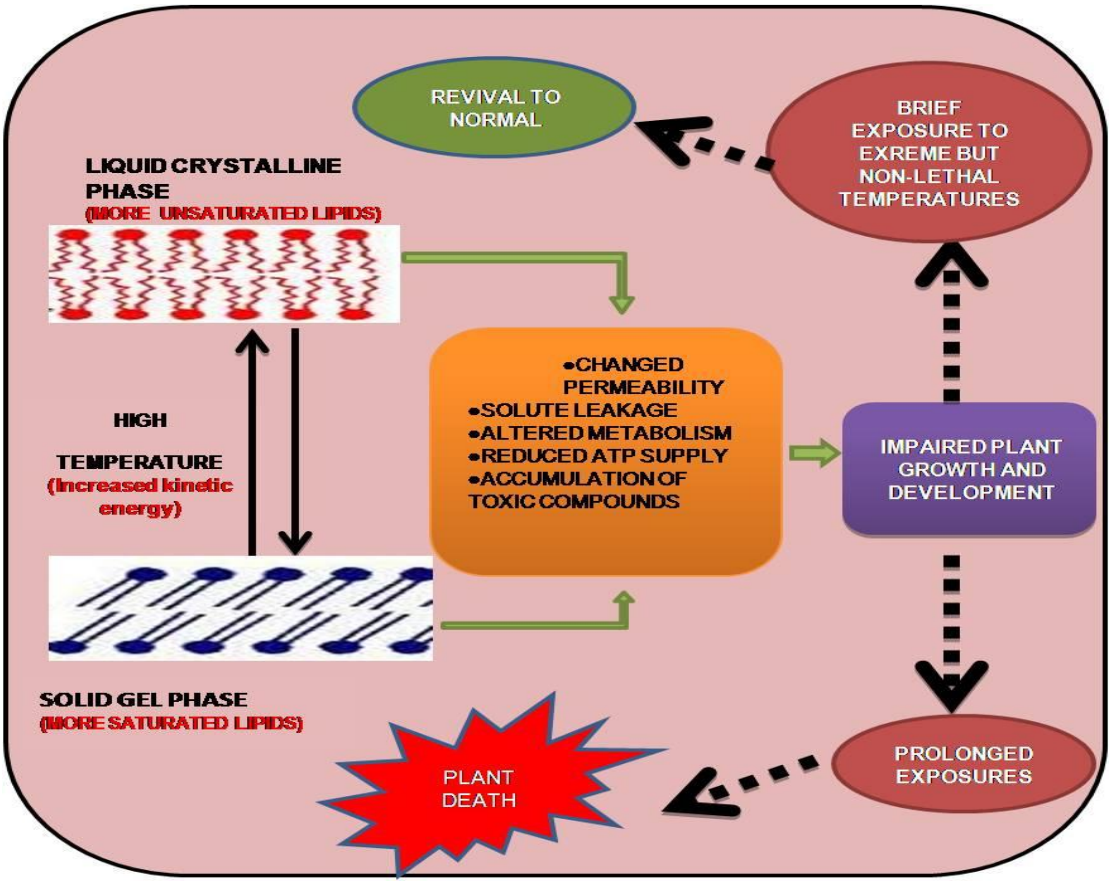

Fig. 4: Summary of temperature stress-induced membrane phase transition and its implications. When exposed to any temperature transition, above or below the normal range, bio-membranes undergo various cellular changes leading to impaired growth and development. The fate of the plant is decided by the intensity and duration of the stressful temperature exposure. If the exposures are brief, injuries may be reverted, otherwise, they may prove fatal to the plant.

4. EFFECTS AT THE METABOLIC LEVEL

4.1 PHOTOSYNTHESIS

As photosynthesis is the vital source of basic carbohydrates and other macromolecules, which are the building blocks for complex carbohydrates, proteins and lipids, any changes in photosynthetic rates will have implications on the overall performance of the plant (Loomis and Connor 1992). The rate of photosynthesis varies in different plant species and is temperature dependent (Cunningham and Read 2002; Hikosaka et al. 2006) and the primary site of photosynthetic damage is PSII (Havaux and Strasser 1992). Low temperatures and high light

bring about photo-oxidation of the photosynthetic machinery in chickpea (Nayyar et al. 2005a,b,c,d) which impairs electron transport, deactivates RUBISCO and decreases stomatal conductance resulting in reduced CO_2 assimilation (Allen and Ort 2001). Low temperature affects the activity of enzyme ribulose activase (RCA), changes the availability of large and small subunits of RUBISCO, disrupts the oxygen-evolving complex (OEC) of PSII and damages the structure and functioning of polypeptides D1 and D2 of PSII (Aro et al. 2000). While studying temperature sensitivity in two pea cultivars, Georgieva and Lichtenthaler (2006) found that chlorophyll fluorescence and the chl/car ratio decreased while the chl a/b ratio increased due to cold stress. Photosynthesis declined in soybean by more than 50% when subjected to only one night of chilling treatment (Van Heerden and Krüger 2000; Van Heerden et al. 2003b). Chilling-induced impaired photosynthesis was further confirmed in soybean (Van Heerden and Krüger 2002; Board and Kahlon 2011) and common beans (Melo et al. 1997; Tsonev et al. 2003). Chilling shock (5°C for 1 day) in broad bean (*Vicia faba*) injured plants thus reducing growth due to loss of chlorophyll and impaired photosynthetic machinery (Hamada 2001). The JIP test measures fast fluorescence transients (Strasser and Strasser 1995) and hence gives a clear idea about the efficiency of photosynthetic machinery, especially PSII function. The JIP test is sensitive to environmental changes and can indicate stress in plants before symptoms appear on the leaves (Christen et al. 2007). When conducted on beans, this test revealed that cold-induced photosynthetic machinery damage was due to impeded electron transport (Goltsev et al. 2010).

An initial increase in temperature may increase photosynthetic activity but prolonged exposure above the normal growth temperature range inhibits photosynthesis (Schuster and Monson1990). Supra-optimum temperatures result in deterioration of photosynthetic pigments (Kumar et al. 2013) and damaged photosynthetic machinery especially the thylakoid lamellae (Hamada 2001; Tambussi et al. 2004). Heat-sensitive bean and chickpea genotypes, upon exposure to stressful high temperatures, had lower chlorophyll content than tolerant genotypes (Petkova 2007; Kumar et al. 2013). Likewise, elevated temperatures reduced the performance of common bean genotype ‘Kentucky Wonder’ more than ‘Haibushi’ owing to impaired photosynthetic machinery (Omae et al. 2006). Even short exposure to temperatures above 40°C disrupted the normal functioning of PSII and impaired the structure and functioning of related proteins and enzymes in soybean (Board and Kahlon 2011) and bird’s foot trefoil (*Lotus japonicus*; Sainz et al. 2010). Heat injury to photosynthetic machinery can be evaluated by measuring chlorophyll fluorescence

parameters, which decreased remarkably in heat-sensitive common bean genotypes over tolerant genotypes (Petkova et al. 2007). Photosynthetic efficiency was evaluated for common bean genotypes using the JIP test for temperature sensitivity (Goltsev et al. 2010) and high temperatures proved detrimental to photosynthetic reaction centers.

The studies carried out by Kumar et al. (2013) on the response of chickpea genotypes to heat stress also corroborated the heat sensitivity of photosynthetic machinery which correlated with a severe reduction in growth and yield (Kaushal et al. 2013). However, there are some reports where, under moderately high temperature (35°C), the functional potential of the photosynthetic apparatus is preserved, e.g. in pea (Haldimann and Feller 2005). The photosynthetic rate and chlorophyll fluorescence have the potential to be used for screening large numbers of genotypes for heat or cold tolerance in food legumes.

4.2 RESPIRATION

Respiration in plants is a temperature-sensitive process (Raison and Lyons 1986) and its rate increases in response to chilling (Sonoike 1999; Kaur et al. 2008). A 68% increase in cellular respiration was reported in chickpea (Nayyar et al. 2005b) up to a certain temperature, beyond which respiration starts to decrease, possibly due to changes in mitochondrial structure, less kinetic energy and impaired structure and function of some important housekeeping proteins and enzymes (Lawrence and Holaday 2000; Munro et al. 2004). In some cases, respiration rates continue to be elevated even when cold exposure ends; this has been attributed to irreversible changes to the metabolic machinery and generation of some high energy intermediates (Steward et al. 1990; Yadegari et al. 2008). Under low temperature, the conventional cytochrome pathway of electron transport is inhibited (Reyes and Jennings 1997) such that, to improve the respiration rate, plants shift to alternative respiration pathways (Gonzalez-Meler et al. 1999; Ribas-Carbo et al. 2000). The two energy-consuming alternative pathways involve AOX (Alternative Oxidase) (Purvis and Shewfelt 1993; Calegario et al. 2003) and PUMP (Plant Uncoupling Mitochondrial Protein) (Vercesi 2001; Calegario et al. 2003). The involvement of alternative pathways is evident from the increased levels of AOX protein and cyanide-resistant respiration in the mitochondria (Vanlerberghe and McIntosh 1992; Gonza`lez-Meler et al. 1999), as observed in fully-grown leaves of cold-stressed mungbean and pea (Gonzalez-Meler et al. 1998, 1999).

In response to high temperatures, respiration is more sensitive than photosynthesis and, in response to an initial increase in temperature, increases exponentially but later plunges significantly beyond a certain limit (Hasanuzzam et al. 2013). Decreased respiration under high temperature has been reported in chickpea (Kumar et al. 2013) and was most likely due to impaired structure and function of mitochondria and proteins, and the effect on the electron transport rate, as reported in some non-leguminous crops such as rice (*Oryza sativa*; Mohammed and Tarpley 2009), tomato (Sato et al. 2000) and turfgrasses (*Festuca arundinacea* L., *Poa pratensis* L.; Jiang and Huang 2001).

5. EFFECTS AT ULTRASTRUCTURAL LEVELS

Damage to cellular components at ultrastructural levels has been well researched (Kratsch and Wise 2000). The extent of damage depends on the relative sensitivity of the various organelles and the severity and damage of the temperature stress (Lee et al. 2002). Chilling injury is well demonstrated at ultrastructural levels and, on the basis of chilling experiments conducted by Wilson (1987) on pea and beans, various organelles can be arranged in decreasing order of chilling sensitivity: plastids > mitochondria > peroxisome > nuclear envelope > tonoplast > plasmalemma. Chilling-induced structural aberrations were noticed in mungbean (Ma et al. 1999) and thought to have manifested in the form of fewer and smaller starch granules and the vesiculation of the inner chloroplastic membrane leading to the formation of peripheral reticulum. If unfavorable low temperatures continue to prevail, the chloroplast may further deteriorate by developing lipid granules, reducing or eliminating starch granules, unstacking grana, disintegrating membranes and even intermixing of chloroplastic and cytoplasmic content as observed in cultured mungbean cells (Ishikawa 1996). Likewise, chilling-inflicted ultrastructural damage in mitochondria was reported as swelling and vacuolation, membrane vesiculation, enlarged and deformed cristae or loss of cristae, membrane disruption or even complete loss of its contents thereby becoming transparent and resulting in intermixing of mitochondrial and cytoplasmic content (Kratsch and Wise 2000; Lee et al. 2002). In cultured mungbean cells subjected to chilling, nuclei shape distorts, the nuclear envelope expands, chromatin material condenses, fibrillar and dense material accumulates in the nucleoplasm and cytoplasm (Ishikawa 1996). Other deformities include cells losing their shape and swelling, disrupted plasmalemma, disorganized vacuolar and mitochondrial membranes, enlarged golgi vesicles, increased vesiculation of ER, disorganization of cytoskeleton resulting in disruption of

vacuoles and/or complete disappearance of vacuoles leading to the breakdown and digestion of organelles. Chilling-induced aberrations in the cell wall and plasma membrane have been reported in mungbean (Yamada et al. 2002). Chilling-stress (28°C dark grown) irreversibly etiolated plants indicating damage to chloroplasts at the ultrastructural level due to suppression of 12 chloroplast-related *cos* genes (Yang et al. 2005). This has not been reported in heat-stressed legumes, however, serious damage at the ultrastructural level in other crops has been reported e.g. damaged chloroplasts in turfgrass (Xu et al. 2006) and damaged mesophyll tissue, disorganized cristae and emptied mitochondria in grapes (*Vitis vinifera*; Zhang et al. 2005) and maize (*Zea mays*; Karim et al. 1997). Exploring the effects of heat stress on ultrastructure in legumes to determine the extent of damage at the organelle level would be useful with regard to the mechanisms associated with heat tolerance.

6. IMPLICATIONS FOR SYMBIOTIC NITROGEN FIXATION

Legumes are economically important as they fix nitrogen in symbiotic association with several rhizobium species to increase soil fertility and decrease reliance on nitrogen fertilizers and, hence, minimize environmental and socio-economic hazards due to the indiscriminate use of fertilizers (Zahran 1999). The legume–rhizobium symbiosis contributes to about 40% of the biologically-fixed nitrogen (Yadav 2008); however, the nitrogen-fixing ability of various bacterial strains is susceptible to numerous environmental constrains (Alexandre and Oliveira 2013; Niste et al. 2013) such as pH and temperature (Fig. 5). The optimum temperature range for nitrogen fixation and nodulation is 20–30°C. Therefore, both high and low temperatures interfere with nodule initiation and development (Al-Falih 2002; Niste et al. 2013). Symbiotic bacteria require optimal temperatures for nitrogen fixation and sub-optimal temperatures impair nodulation, prolong root infection and impede nodule development and nitrogenase activity (Drouin et al. 2000). Under low temperatures, the production and secretion of Nod factor by *Rhizobium leguminosarum* bv. *trifolii* decreased, which retarded growth in alfalfa (Rice and Olsen 1988) and soybean (Lynch and Smith 1993). In soybean, low temperature hindered infection and early nodulation, reduced (or complete loss of) N_2-fixation as the temperature dropped from normal (25°C) to 15°C (Zhang et al. 1995). The threshold temperature for marked nodulation interruption is legume specific e.g. common beans and soybean have a similar threshold whereas lentil is more tolerant such that only temperatures below 10°C substantially diminish nodulation (Lira et al. 2005). The findings of Cloutier et al. (1992) on the cold-shock

response in temperate rhizobia such as *Sinorhizobium meliloti* and arctic rhizobium species indicated that, similar to other bacteria, rhizobia also generate various heat shock proteins (HSPs) and cold-shock proteins (CSPs). Further studies on the cold-shock operon in *S. meliloti* revealed the occurrence of *S. meliloti* cspA cold-shock operon similar to *E.coli* cspA. The cspA protein thus produced in *S. meliloti* acts as an RNA chaperone i.e. maintains mRNA translation even under cold conditions (O'Connell and Thomashaw 2000). Likewise, Drouin et al. (2000) studied cold adaptation in *R. leguminosarum* bv. *viciae* (microsymbiont of *Lotus japonicas*) that can grow at 5°C and noted the occurrence of higher molecular weight CSPs in these strains. Similarly, Sardesai and Babu (2001) studied cold tolerance in *Rhizobium* DDSS69; it was thought that the cold alleviation by DDSS69 was due to two high molecular weight polypeptides (135 and 119 kDa) detected within 15 min exposure of *Rhizobium* to 5°C (Sardesai and Babu 2001; Phadtare et al. 2000). The CSPs generated by cold-tolerant rhizobia bind to nucleic acids, maintaining translation and transcription at low temperatures. The rate of symbiotic nitrogen fixation in two soybean genotypes differing in sensitivity to chilling reduced under cold stress as both genotypes fixed less nitrogen. Nodule respiration, nitrogenase activity and NifH and nifK mRNA were also inhibited in these genotypes. When reverted back to optimal temperature, the cold-tolerant genotypes recovered nodule respiration unlike the sensitive genotype, thereby, fixing limited N_2 and reducing nitrogen availability to the plant (Van Herdeen et al. 2008). In an earlier study, the effects of dark chilling were curbed in the presence of nitrogen (Van Heerden et al. 2004). It is evident that chilling-induced inhibition primarily targets symbiotic nitrogen fixation and the results concurred with earlier reports (Van Herdeen et al. 2004; Strauss et al. 2007).

While temperatures below 10°C result in poor nodulation as well as rhizobial growth, some strains of Rhizobia and Bradyrhizobia, particularly from arctic and sub-arctic regions, are adapted to temperatures as low as 4°C (Van Heerden et al. 2004). These well-adapted rhizobia are markedly competitive and could be used to improve symbiotic nitrogen fixation and thus the yield of legumes grown under cold conditions (Prévost et al. 1999). For example, *Bradyrhizobium japonicas* isolated from cold soils in Japan effectively ameliorated seed yield and nitrogen-fixing traits in soybean (Lynch and Smith 1993). Nitrogenase activity improved and shoot dry matter increased in the temperate legume sanfoin (*Onobrychis viciifolia*) inoculated with rhizobial strains indigenous to Canadian high arctic (Prévost et al. 1999) Therefore, cold-

adaptive strains of various symbiotic bacteria may be exploited for legume cultivation under low temperature. Compared to studies on cold stress, the impact of heat stress on rhizobia has been thoroughly studied (Kulkarni and Nautiyal 1999; Zahran 1999; Lira et al. 2005).

The effect of high-temperature exposure on nodulation and the efficiency of nitrogen fixation in common beans has been investigated (Hungria et al. 1993); plants in the high-temperature treatment (35 and 38°C/8 h/day) formed nodules, but these nodules were inefficient at fixing nitrogen. The control plants (grown at 28°C), when exposed to even higher temperatures (40°C/8 h/day) at the flowering stage, displayed a substantial reduction in nitrogenase activity and nitrogen-fixation efficiency. No nodules formed in peanut at 40°C or soybean at 37°C; the maximum temperature range for rhizobial growth is 32–47°C (Hungria and Vargas 2000). It was further established by Rahmani et al. (2009) that heat tolerance of *Bradyrhizobium* directly affects the symbiotic efficiency between the bacterium and host soybean and all stages of legume–rhizobium symbiosis are susceptible to high temperature (Hungria and Vargas 2000; Yadav and Nehra 2013). In mungbean, beneficial effect of inoculation with high temperature tolerant rhizobial isolates was more pronounced at higher (37-49°C) temperature regime (Bansal et al. 2014).Therefore, high-temperature-tolerant nitrogen-fixing rhizobial strains may serve as an efficient intervention in mitigating temperature stress in crop plants (Yadav and Nehra 2013). The correlation between thermo-tolerance and nitrogen-fixation efficiency of a rhizobial strain has been demonstrated in studies worldwide e.g. *Bradyrhizobium* strains capable of surviving at 42°C showed efficient nitrogen-fixation even under high temperatures (Kishinevsky et al. 1992) which is similar for *Rhizobium* (Michiels et al. 1994; Nehra et al. 2007). Tolerant strains of *Mesorhizobium*, when exposed to heat shock, had higher GroEL (HSP60) gene expression compared to susceptible strains (Laranjo and Oliviera, 2011). The GroEL (HSP60) is an HSP that acts as a chaperone in maintaining the structure and folding of various proteins (Lin and Rye 2006; Horwich and Fenton 2009). These findings were in accordance with the findings of Alexandre and Oliviera (2011) in chickpea thus indicating some heat-adaptive response. Similarly, the over-production of HSP GroEL under heat stress was detected in chickpea *Mesorhizobia* (Rodrigues et al. 2006) and arctic rhizobia (Cloutier et al. 1992). In *Bradyrhizobium japonicum* and *Klebsiella pneumoniae*, GroEL was crucial for regulating the nif gene and it also acted as a chaperone (Fischer et al. 1999; Ribbe and Burgess 2001). HSP accumulation in *Rhizobium* under heat stress has been reported in various other studies

(Wallington and Lund 1999; Natera et al. 2000). The proteome profiling of *Rhizobium tropici* strain PRF81 grown at 28 and 35°C revealed up-regulation of about 59 different proteins under heat stress including HSPs such as DnaK and GroEL, along with various anti-oxidative proteins indicating some cross-talk between heat and oxidative stresses (Gomes et al. 2012). Some studies have contradicted the positive correlation between temperature tolerance and symbiotic nitrogen fixation (SNF) where either no correlation was observed, as reported in *Acacia nilotica* (Rustogi et al. 1996) and in *Bradyrhizobium* and *Rhizobium* (Gopalakrishnan and Dudeja 1999) or a negative correlation was reported, as observed in lentil (Moawad and Beck 1991).

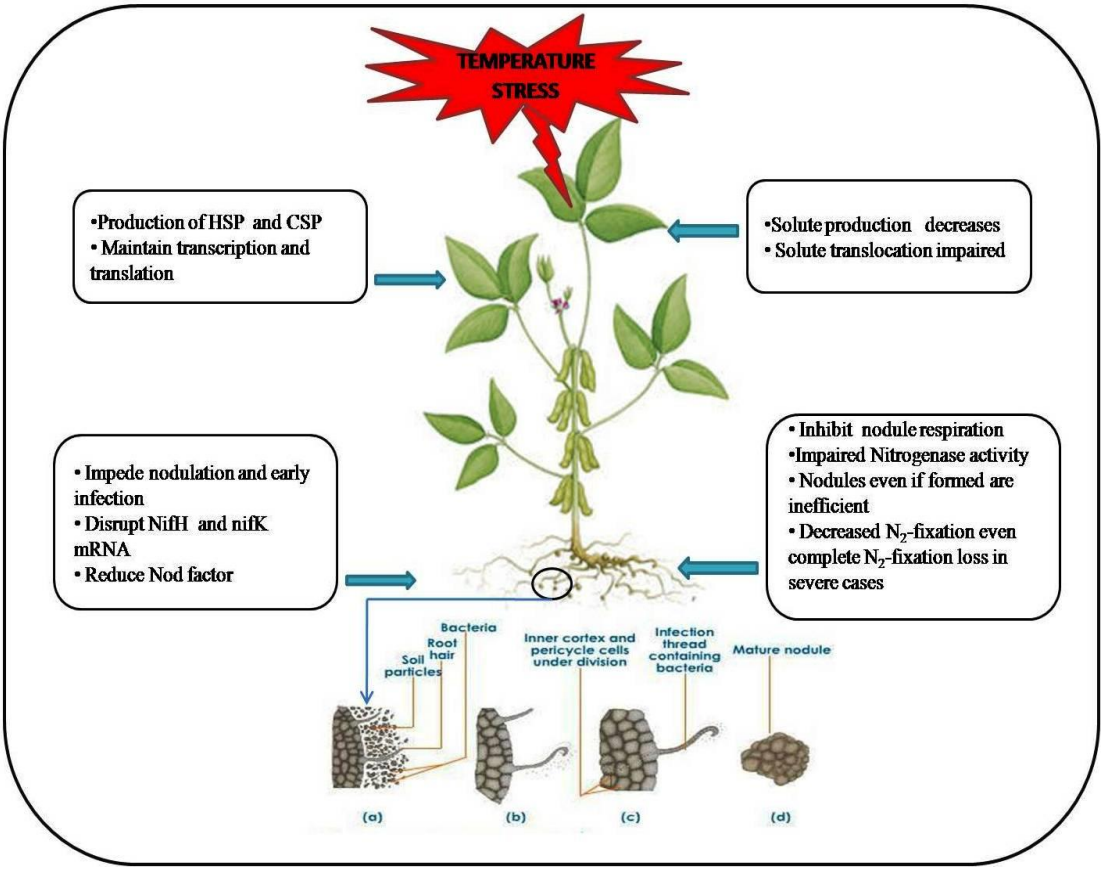

Fig. 5: Effect of temperature on symbiotic nitrogen fixation. Unfavorable temperatures (high and low) impair nodulation and nitrogen fixation resulting in less solute production and transportation and the production of various heat- and cold-shock proteins.

7. TEMPERATURE-INDUCED OXIDATIVE STRESS

Abiotic stresses alter the normal metabolic functioning of plants which have developed ways to adjust to stress conditions including enhancing anti-oxidative machinery (Shaulev et al. 2008), recently reviewed by us in temperature-stressed crops (Awasthi et al. 2015). Reactive oxygen species (ROS) are normally produced as byproducts of various cellular oxidation processes (Finkel and Holbrook 2000; Perl-Treves and Perl 2002) and act as useful secondary messengers (Gechev et al. 2006; Yan et al. 2006). Therefore, ROS help plants to adapt to a wide range of stresses by maintaining cellular homeostasis, controlling transcription and translation, and maintaining energy supply and protein phosphorylation (Mittler et al. 2011); however, there is a delicate balance between ROS generation and its scavenging and over-production can be harmful (Fig. 6; Esfandiari et al. 2007).

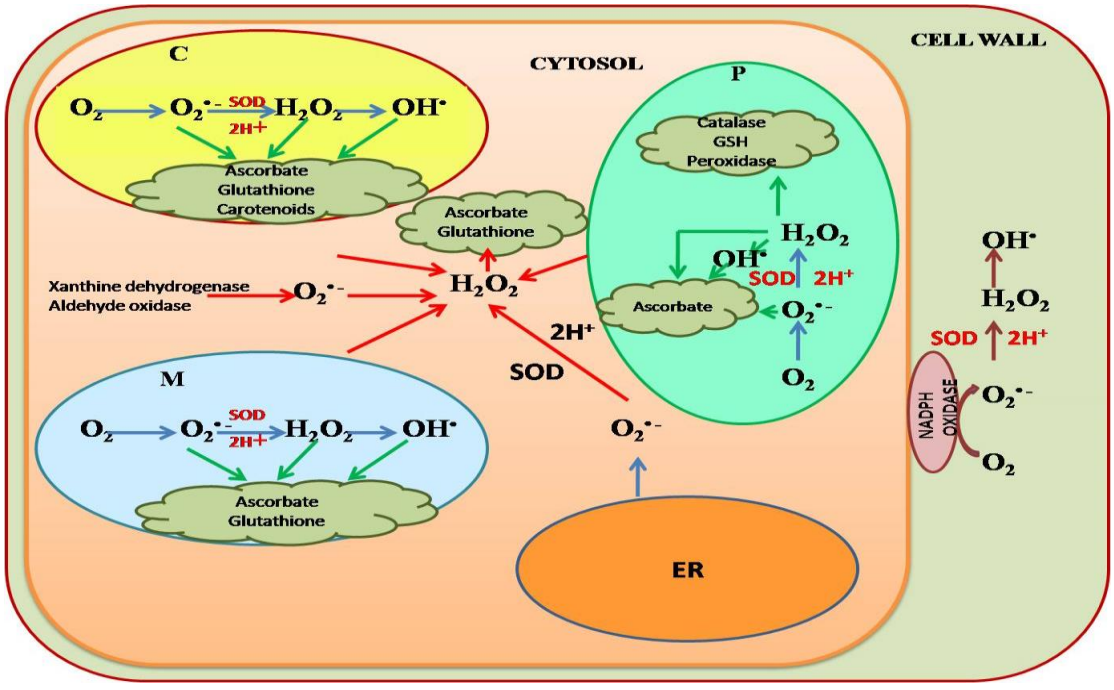

Fig. 6: Generation and scavenging of various reactive oxygen species in a plant cell. In response to various abiotic stresses including temperature, ROS are generated at numerous cellular sites through specific reactions. When the ROS levels increase beyond a certain limit, ROS quenching by enzymatic and non-enzymatic antioxidants comes into play to maintain

cellular homeostasis (M, mitochondria; C, chloroplast; P, peroxisome; ER, endoplasmic reticulum; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; SOD, superoxide dismutase; GSH, reduced glutathione; $O_2^{\bullet}$, superoxide radical; $OH^{\bullet}$, hydroxyl radical; H_2O_2, hydrogen peroxide; O_2, oxygen).

ROS is generated at various sites in the cell such as in chloroplasts (Foyer and Harbinson 1994; Hormann et al. 1994), mitochondria (Noctor et al. 2007; Rasmusson et al. 2008), endoplasmic reticulum, peroxisome and glyoxisomes (Mittler 2002; Noctor et al. 2002). Increased and uncontrolled generation of ROS during stress may be lethal to plants as it can lead to lipid peroxidation, oxidative denaturation of proteins and enzymes, impairment of nucleic acids and even plant death under prolonged stress exposures (Mittler 2002; Mishra et al. 2011; Srivastava and Dubey 2011). Each plant has its own anti-oxidative system where ROS-scavenging enzymes such as SOD (superoxide dismutase), CAT (catalase), APO (ascorbate peroxidase), POX (peroxidase) and GR (glutathione reductase) come into play (Fig. 6) along with non-enzymatic antioxidants such as glutathione (GSH), ascorbate (AsA) and carotenoids (Gill and Tuteja 2010; Hasanuzzaman et al. 2012). ROS quenching by anti-oxidative machinery is linked to stress tolerance e.g. higher cold tolerance was observed in plants with enhanced activities of anti-oxidative enzymes in chickpea (Kumar et al. 2011b) and alfalfa (Wang et al. 2009). Soybean seedlings exposed to very low-temperature treatments (1°C) increased activities of anti-oxidative enzymes (Posmyk et al. 2005). Similarly, Kaur et al. (2008) reported increased activity of anti-oxidative enzymes in chickpea pod walls to protect pods and developing seeds from chilling injury. The chilling experiments carried out by Wang et al. (2009) on alfalfa genotypes with different chilling sensitivities showed that the chilling-tolerant genotypes had high anti-oxidative activity over the chilling-sensitive genotypes. Likewise, Turan and Ekmekçi (2011) exposed chickpea cultivars to stressful low temperatures (2 and 4°C) and reported enhanced activities of PSII and anti-oxidative enzymes in acclimated plants.

ROS generation has been reported under heat stress (Potters et al. 2007) and is an indicator of cellular damage due to lipid peroxidation and altered membrane permeability. Under high temperatures, a plant's anti-oxidative system gears up for easing heat-induced oxidative stress as observed in chickpea (Kaushal et al. 2011; Kumar et al. 2013), soybean (Djanaguiraman et al. 2011), bird's foot trefoil (Sainz et al. 2010), horse gram (*Macrotyloma uniflorum*; Naji and Devaraj 2011), mungbean (Mansoor and Naqvi 2013) and lentil (Chakraborty and Pradhan

2011). Chickpea plants exposed to 45/40°C (day/night) showed varied expression of enzymatic (SOD, CAT, APX, GR) and non-enzymatic antioxidants (AsA, GSH) (Kaushal et al. 2011). Lipid peroxidation and H_2O_2 (hydrogen peroxide; an oxidative molecule) levels were higher at high temperature, i.e. at 40/30°C, which further increased when the temperature reached 45/35°C. Simultaneously, the content of enzymatic and non-enzymatic antioxidants also increased at 40/30°C but decreased with any further increase in temperature. Similar observations were recorded by Chakraborty and Pradhan (2011) in their heat treatment experiments on lentil, where tolerant varieties had higher anti-oxidative properties than sensitive ones when exposed to high temperatures (35–45°C). Mungbean exposed to stressful high temperatures (50°C for 2 h) also expressed enhanced levels of anti-oxidative enzymes such as CAT, POD, SOD and APO in thermo-tolerant genotypes (Mansoor and Naqvi 2013). Ascorbic acid (Kumar et al. 2011) and glutathione (Nahar et al. 2015) applications to heat-stressed mungbean decreased the oxidative damage by activating various enzymatic and non-enzymatic antioxidants.

8. OSMOLYTES AND PHYTOHORMONES UNDER TEMPERATURE STRESS

Of all the molecules in cells affected by temperature stress, osmolytes and phytohormones have drawn the most attention. Endogenous levels of these molecules change to varying degrees in response to stress as part of a plant's response to stress and possibly as a protection mechanism against the adverse conditions. Moreover, their exogenous application has been beneficial in ameliorating plant performance under stressful conditions. Previously, we have reported on the roles of various cryoprotectants involving osmolytes and phytohormones (Bhandari and Nayyar 2014); here we discuss their involvement in response to temperature stress and their potential application in improving stress tolerance.

8.1 PROLINE

Proline is an amino acid with known osmoprotective functions under a wide range of abiotic stresses (Hare et al. 1999, Ashraf and Foolad 2007) including temperature (Jonytiene et al. 2012; Yang et al. 2012). Proline maintains the redox potential of cells by regulating the NAD^+/NADH ratio (Verbruggen and Hermans 2008; Aghaee et al. 2011) which stabilizes the structure and function of biomembranes, enzymes and proteins (Zhang et al. 2011). Proline is effective in scavenging various ROS, especially singlet oxygen, by forming stable compounds (Szabados and

Savoure 2009; Aghaee et al. 2011) and maintaining nitrogen fixation under stress (Kohl et al. 1988). Additionally, proline may act as a potential secondary messenger under stress (Szabados and Savoure 2009; Hayat et al. 2012). The endogenous levels of proline were up-regulated in cold-stressed soybean (Yadeghari et al. 2008) and chickpea (Kumar et al. 2010) under low and freezing temperatures. In chickpea (Kumar et al. 2010) and *Medicago* spp. (Zhang et al. 2011), such an increase was positively correlated to cold tolerance. In mungbean, an exogenous supply of proline during seed priming alleviated chilling injury (Posmyk and Janas 2007).

Heat-sensitive chickpea genotypes accumulated less proline than tolerant genotypes (Kumar et al. 2013) suggesting a vital role for proline in heat tolerance. In cowpea, reduced proline levels under heat stress resulted in impaired pollen development (Mutters et al. 1989; Tang et al. 2008) indicating a role for proline in maintaining pollen viability (Lansac et al. 1996). Exogenous application of proline helped to mitigate heat stress in chickpea by maintaining the efficiency of anti-oxidative machinery and carbon assimilation enzymes even under elevated temperatures (Kaushal et al. 2011). Transgenic soybean plants which overproduce proline have been developed (de Ronde et al. 2000, 2004) and show heat tolerance.

8.2 GLYCINE BETAINE

Glycine betaine (GB) is an important quaternary ammonium compound that accumulates to high levels under various abiotic stresses (Ashraf and Foolad 2007; Giri 2011). GB protects the photosynthetic machinery from photo-inhibition induced damage by protecting the OEC of PSII and maintaining the generation of proteins required for repairing damaged PSII (Chen and Murata 2011; Giri 2011). GB also maintains membrane integrity (Gorham 1995; Hamilton and Heckathorn 2001), stabilizes membrane structure and, hence, the functioning of various enzymes and proteins by maintaining a hydration envelope (Sakamoto et al. 2000; Chen and Murata 2011). It also eases oxidative damage by activating the anti-oxidative system of the plant (Chen et al. 2000; Giri 2011). Under cold stress, GB decreased markedly in cold-stressed chickpea, which was related to reproductive failures (Nayyar et al. 2005d). Exogenous application of GB not only increased pollen viability, pollen tube growth, stigma receptivity and ovule viability at bud stage but also increased plant biomass and yield. Similar cryoprotective effects of exogenously-applied GB were reported in *Medicago* seedlings (Zhao et al. 1992). *In vivo* and *in vitro* application of GB increased pollen germination by 15 and 21%, respectively, in several

soybean genotypes tested under high temperatures (Salem et al. 2005). More recently, limited work has been conducted on GB in temperature-stressed legumes; it would be worthwhile to examine its role under low and high temperature stress.

8.3 SALICYLIC ACID

Salicylic acid (SA) refers to a group of widely-found plant phenolics which have hormone-like functions (Kang et al. 2003). SA plays an important role in numerous physiological functions (Shakirova et al. 2003), particularly in defense mechanisms against various biotic and abiotic stresses (Yalpani et al. 1994; Szalai et al. 2000) including temperature extremes (Mabood and Smith 2007). SA leads to stress mitigation through the fortification of anti-oxidative machinery and membrane stability (Qu et al. 2013), thereby improving plant performance at stressful high temperatures (Sakata et al. 2010). SA also helps to initiate and maintain legume–rhizobia symbiosis particularly during the initial stages of the plant–rhizobium interaction (Mabood and Smith 2007). Bean plants initially subjected to chilling (0±0.5°C for 2 days) followed by heat stress (54±0.5°C for 3 h) treated with SA or acetylsalicylic acid (ASA) showed 100% survival compared with untreated plants due to improved anti-oxidative machinery. Similar findings were reported for pea (Srivastava and Dwivedi 1998) and suggested by Chen et al. (1997) in rice. Results were validated further in heat-stress experiments in chickpea where SA had beneficial effects in mitigating heat-induced injury by improving membrane stabilization, increasing proline and protein levels, and increasing the activity of anti-oxidative enzymes such as POX and APX (Chakraborty and Tongden 2005). Endogenous SA levels need to be correlated with adverse temperatures to provide better insight about its role in stress situations.

8.4. POLYAMINES

Polyamines (PAs) are low molecular weight, ubiquitous, nitrogenous organic polycations, which perform profound biological functions (Capell et al. 2004; Kuznetsov et al. 2006). Biologically-important PAs are diamine putrescine (Put), triamine spermidine (Spd) and tetraamine spermine (Spm; Kaur-Sawhney et al. 2003; Kuznetsov et al. 2006). Of the total PA content in cells, 2–30% occurs in a bound state (bound to low- or high-weight molecules: phenolic acids, proteins, nucleic acids, membrane structures; Martin-Tanguy 2001; Kaur-Sawhney et al. 2003) with the remainder (70–98%) in a free state (Martin-Tanguy 2001; Kaur-Sawhney et al. 2003). PAs help to mitigate various stresses (Kusano et al. 2008; Alcàzar et al. 2010,) which is likely due to their

involvement in signal transduction pathways operating under different stresses (Vinocur and Altman 2005; Alcàzar et al. 2006). The protective functions of PAs have been attributed to their anti-oxidative properties, cell wall stabilizing ability and acid neutralizing capacity (Zhao and Yang 2008). Transgenics over-expressing polyamine biosynthesis genes tolerate various abiotic stresses including temperature (Kasukabe et al. 2006; Prabhavathi et al. 2007; Wen et al. 2008). The role of PAs under low temperature stress has been reported in many studies (Yoshikawa et al. 2007; Cuevas et al. 2008; Groppa and Benavides 2008; Alcázar et al. 2010). Enhanced endogenous PA levels were reported in *Arabidopsis thaliana* subjected to both sub and supra-optimal temperatures (Todorova et al. 2007) and in wheat genotypes under heat stress (Goyal and Asthir 2010). Chilling-tolerant plants had higher PA levels than relatively sensitive plants in *Phaseolus* spp. (Guye et al. 1986) and chickpea (Nayyar and Chander 2004; Nayyar 2005). Exogenous PA treatment alleviated cold-induced oxidative stress in chickpea by increasing endogenous putrescine, which reduced hydrogen peroxide (H_2O_2), malondialdehyde concentration and increased the levels of enzymatic and non-enzymatic antioxidants (Nayyar and Chander 2004). Supplementation with exogenous PAs helped to maintain yield and other yield-related attributes in winter-sown chickpea under cold-stressed conditions (Nayyar 2005). The role of PAs in heat-stressed legumes has not been studied and needs investigation.

8.5. NITRIC OXIDE

Nitric oxide (NO), previously considered an air pollutant, is now recognized as a signaling molecule which affects various physiological processes ranging from seed germination**,** seed dormancy, leaf expansion, plant maturation and senescence (Mishina et al. 2005), floral transition (He et al. 2004), ethylene emission and stomatal closure (Garcia-Mata and Lamattina 2007) to even programmed cell death, light-mediated greening and the regulation of responses to various abiotic and biotic stresses (Uchida et al. 2002; Bethke et al. 2007; Floryszak-Wieczorek et al. 2007). Numerous studies support the anti-oxidative role of NO as it may act as a signaling molecule triggering the activities of various anti-oxidative enzymes. It may be directly or indirectly associated with other signaling molecules such as H_2O_2, SA and cytosolic Ca^{2+} (Neill et al. 2003; Wendehenne et al. 2004). NO may bring about S-nitrosylation of protein thiols to form S-nitrosothiols (Abat and Deswal 2009); this reversible process of protein S-nitrosation–denitrosation may regulate signal transduction resulting in activation or deactivation of various proteins (Hayat et al. 2010). However, there is no common consensus on the exact mechanism(s)

of abiotic stress amelioration by NO (Siddiqui et al. 2011). Endogenous levels of NO have been elevated in response to short-term heat stress in alfalfa (Leshem 2001) and to low temperatures in bird's foot trefoil (Shimoda et al. 2005) and pea (Corpas et al. 2008) suggesting its possible involvement in temperature stress responses. Application of exogenous NO in the form of SNP (sodium nitroprusside; NO donor) during heat shock in mungbean maintained photosynthetic machinery stability, membrane integrity and improved the anti-oxidative defense (Yang et al. 2006). It also improved the chlorophyll concentration in pea (Leshem et al. 1997) and ameliorated heat shock damage in mungbean leaves (Yang et al. 2006). NO may interact with plant hormones to influence the stress response. The involvement of NO in ABA-induced improvement in anti-oxidative defenses in chilling-stressed Brazilian lucerne (*Stylosanthes guianensis*/*Trifolium guianense*) has been observed (Zhou et al. 2005). Further research on the role of NO in temperature-stressed plants is needed.

8.6. ABSCISIC ACID

ABA plays a vital role in plants especially under stress conditions (Swamy and Smith 1999). It regulates stomatal closure, shoot growth, leaf senescence (Swamy and Smith 1999), seed germination, seed dormancy (Finkelstein et al. 2008) and signal transduction (Park et al. 2009). When plants experience environmental stress such as cold or heat, various cellular responses occur such as increases in endogenous ABA levels leading to activation of Ca^{2+} which mobilizes secondary messengers such as cADPR (Campalans et al. 1999) and inositol triphosphate (IP_3)., as seen in broad beans (De Wald 2001) and *Arabidopsis* seedlings (Sanchez and Chua 2001; Xiong et al. 2001). Increased Ca^{2+} levels trigger a transcriptional cascade involving various transcription factors and genes. The role of ABA in chilling tolerance has been observed in many studies. Elaborate studies on ABA mutants of alfalfa confirmed the role of ABA in chilling tolerance (Mohapatra et al. 1988; Penna-Cortis et al. 1991). Exogenous application of ABA is effective for cold stress mitigation when applied alone (Bakht et al. 2006) or in combination with other compounds such as SA (Szalai et al. 2011), as seen in chickpea (Nayyar et al. 2005a; Kumar et al. 2008). Exogenous supplementation with ABA not only mitigated stress injuries in chickpea by improving pollen viability and germination but also improved yield and oxidative stress defense mechanisms (Kumar et al. 2008). Endogenous ABA levels have reportedly increased under heat stress suggesting its involvement in thermo-tolerance (Robertson et al. 1994; Teplova et al. 2000), which was confirmed in chickpea by Kumar et al. (2012). ABA

689 treatment not only enhanced endogenous ABA levels in chickpea but also improved yield and
690 yield-related attributes such as flower retention, biomass, pod set, seed size and grain yield
691 (Kumar et al. 2008).

692 **8.7 BRASSINOSTEROIDS**

693 Brassinosteroids (BRs) are naturally-occurring steroidal hormones, which were first isolated
694 from pollen grains of *Brassica napus* L. hence the name (Grove et al. 1979). These hormones
695 occur universally in almost every plant part (Bajguz and Hayat 2009). BRs have been implicated
696 in a plethora of physiological effects such as cell division and elongation, pollen tube growth,
697 seed germination and reproductive growth (Sharma and Bhardwaj 2007; Ye et al. 2010), leaf
698 movement, root growth inhibition, vascular differentiation, ethylene synthesis and senescence
699 (Kim et al. 2012), nucleic acids and proteins synthesis, and the activation of various
700 photosynthetic and nitrogen-fixation enzymes (Farooq et al. 2009; Hola 2011). BRs affect plant
701 growth and development either alone or in association with other phytohormones and
702 biomolecules (Gomez et al. 2011). The mechanism of BRs protection has been well studied at a
703 molecular level; BRs are sensed by receptor kinases present on the cell surface and the signal is
704 transduced to the nucleus through a series of intracellular signaling components involving
705 numerous protein–protein interactions which consist of enzymes such as kinases, phosphatases
706 and other proteins like 14-3-3 protein and transcription factors (Codreanu and Russinova 2011).
707 Evidence suggests that BRs also play an important protective role under various abiotic stresses
708 (Vardhini and Rao 2003). The growth of chilling-stressed mungbean epicotyls improved with the
709 application of exogenous 24-BR (Huang et al. 2006), which was confirmed in studies on
710 groundnut (Vardhini and Rao 1998). The beneficial effects of BRs have been reported under
711 both cold stress and heat stress in mungbean (El-Bassiony et al. 2012). Application of BRs
712 improved growth and biomass in these crops under temperature stress thereby substantiating the
713 similar reports by Upreti and Murti (2004) in water-stressed french beans.

714 **9. SCREENING AND BREEDING FOR TEMPERATURE TOLERANCE**

715 In order to cope with ever-fluctuating temperature extremes, to which various legumes are
716 exposed, efforts are being made to develop more tolerant plant varieties. Selection of
717 temperature-tolerant lines is done using various methods. Classically, two criteria have been
718 followed to evaluate the tolerance of generated lines: survival percentage and visual rating.

Yield-related attributes such as the number of filled pods, number of seeds and harvest index can also be used to select better plants (Canci and Toker 2009; Gaur et al. 2015). Additionally, pollen-based screening marker-assisted selections along with gametophytic selection and precise phenotyping can be employed for better temperature stress tolerance evaluation (Clarke et al. 2004; Gaur et al. 2015).

Using wild relatives of various cool-season legumes in breeding experiments is one strategy to address cold tolerance (Meuhlbauer et al. 1994). Wild species have been collected from their supposed centers of origin (Van der Maesen and Pundir 1984; Meuhlbauer et al. 1990) to serve as a potential genetic source of tolerance genes to various abiotic and biotic stresses. However, some classical plant breeders emphasize the need to first use the genomes of already-cultivated crops (Hawtin et al. 1988). Nevertheless, full use of wild germplasm has been limited due to the crossability barrier, the basis of which Harlan and De Wit (1971) divided various wild species into primary, secondary and tertiary gene pools (Table 4). The primary gene pool consists of species which can freely interbreed and produce fertile progenies while interbreeding is limited in the secondary gene pool and hybrids are less fertile. In tertiary gene pool, intercrossing is not feasible and, if carried out, the progeny is sterile.

Table 4: Primary, secondary and tertiary gene pool of some important food legumes (Muehlbauer et al. 1994).

LEGUME CROP	GENE POOLS		
	PRIMARY	SECONDARY	TERTIARY
Chickpea	*Cicer arietinum* *Cicer reticulatum* *Cicer echinospermum*	–	*C. bijugum* *C. pinnatifidum* *C. judaicum* *C. chorassanicum* *C. montbretti*
Lentil	*Lens culinaris* ssp. *culinaris* *Lens culinaris* ssp. *orientalis* *Lens culinaris* ssp. *odemensis*	*Lens nigricans* ssp. *nigricans* *Lens nigricans* ssp. *ervoides*	–
Pea	*Pisum sativum* ssp. *sativum* *Pisum sativum* ssp. *elatius* *Pisum sativum* ssp. *humile*	*Pisum fulvum*	–
Faba bean	*Vicia faba*	–	*V. narbonensis* *V. hyaeniscyamus* *V. galilaea* *V. johannis* *V. bithynica*

Following is an account of the potential genetic sources (Table 5) identified worldwide for cold and heat tolerance for various legumes.

CHICKPEA: It is one of the most important cool-season legumes and is temperature sensitive. Screening of 10,000 chickpea breeding lines and germplasm for cold tolerance revealed ILC 8262 and ILC 482M to be the most resistant. Wild chickpea germplasm, when screened, was cold tolerant and thus can be used in breeding experiments. *C. bijugum, C. judaicum* and *C. pinnatifidum* have been successfully crossed with the cultigen (Verma et al. 1990, 1991). The cold tolerance trait is affected by additive and non-additive gene actions along with other genic interactions (Singh et al. 1994). Many studies have been done to screen for heat-tolerant chickpea genotypes using various parameters such as pollen viability, stigma receptivity and yield. ICRISAT and ICARDA have identified several heat-tolerant genotypes (Table 5) for both desi (ICCV 92944, ICCV 93952, ICCV 96970 etc.) and kabuli (ICCV 95332, ICCV 92318, FLIP 87-59C etc.). Of these, ICCV92944 has been released in developing countries such as India, Myanmar and Kenya with supra-optimum temperature exposures and is being quickly adopted by farmers (Gaur et al. 2015).

COMMON BEAN: It is among the heat-sensitive legumes with yield severely affected by high temperatures. Tepary bean (*Phaseolus acutiflolius* A. Gray) is inherently heat tolerant and thus has been exploited in various breeding experiments at CIAT (International Center for Tropical Agriculture). The derived interspecific lines have recorded higher yields even under high-temperature conditions when compared with common beans (Gaur et al. 2015).

FABA BEANS: These are sensitive to water, cold and heat stress. Studies evaluating winter hardiness and frost tolerance in faba bean genotypes have indicated that traits such as changes in fatty acid composition, ion leakage and free proline content are strongly correlated with frost tolerance (Arbaoui et al. 2008; Link et al. 2010), and hence, may be used for screening tolerant lines. Two heat-tolerant varieties of faba beans (Shendi and Marawi) have been released in Sudan (Table 5; Gaur et al. 2015).

LENTIL: *Lens culinaris* ssp. *orientalis* is considered the best source for winter hardiness (Hamdi et al. 1996) and the progenies thus generated have been listed among the elite lines in Lentil International Trials (Erskine et al. 1994). Various late-sowing experiments have reported

767 heat sensitivity in lentil and a few heat-tolerant genotypes have been identified (Table 5)
768 including IL12181, ILL82, ILL5151, ILL5416 (Gaur et al. 2015).

Table 5: The genetic sources(cultivars/accessions/elite lines/germplasm accessions) identified for cold and heat tolerance in various legumes.

CROP	COLD TOLERANT	REFERENCE	HEAT TOLERANT	REFERENCE
Chickpea	Hybrids of *C. reticulatum*	Singh et al. 1995	FLIP 87-59C	Singh et al. 1996
	C. echinospermum	Malhotra, 1998	FLIP 92-154C	Toker and Cagirgan 1998
	ILC 8262, ILC 8617,		ICCV 92944, ICCV 93952	Gaur et al. 2015
	FLIP 87-82C		ICCV 96970, ICCV 94954	
	SP1.563, Gully, 940-26	O'Toole et al. 2001	ICCV 07102, ICCV 07110	
			ICCV 07109, ICCV 07118	
			ICCV 07117, ICCV 07105	
			ICCV 07108, ICCV95332	
			FLIP 87-59C	
			Salawa, Burguieg	
			S051708, S00998	
			S03308, S03525	
			S051702, S051412	
			S03302, S02266	
			S051685, S051703	
Faba	Côte d'Or, BPL 4628	Duc and Petitjean,1995	Shendi	Gaur et al. 2015
beans	ILB 12, ILB 14	Olszewski, 1996	Marawa	
	ILB318, ILB 3187			
	ILB 2999			
Lentil	LC9978057, LC9977006	Hamdi et al. 1996	ILL2181, ILL 82	Gaur et al. 2015
	LC9977116, LC9978013		ILL 5151, ILL5416	
	ILL759, ILL1878,ILL4400		ILL 4587, ILL 956	
	ILL7155, ILL8146,ILL8611		ILL 598,	
	ILL9832, Kafcas, Cifei, Ubek		FLIP 2009-55L	

	Balochistan local, ILL5865	Ali et al. 1999	ILL 2507, ILL 4248	Gaur et al. 2015
	WA8649041	Kahraman et al. 2004		
	WA8649090			
	ILL1878	Sarker et al. 2002		
	ILL662, ILL857,			
	ILL975, ILL1878			
Pea	EFB33, Unrra	Urbatzka et al. 2005	Arka Ajit	Upreti and Murti, 1999;2000
	Wűrttembergische		Acc. 623, 765	Srikanthbabu et al. 2002

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9.1 MOLECULAR APPROACHES AND TRANSGENICS

Conventional plant breeding methods often transfer undesirable donor DNA segments which may be harmful and need to be removed (Vogan and Giggs 2011). Thus, a system based on foreground and background selection involving molecular markers and linkage maps was devised to minimize transfer of undesirable genes. Genetic linkage maps have been developed for various cool-season legumes such as pea (Weeden and Wolko, 1990; Ellis et al. 1992), lentil (Harry and Muehlbauer 1989; Weeden et al. 1992; Simon et al. 1993) and chickpea (Simon and Muehlbauer 1991, Flandez-Galvez et al. 2003).With the revolutionary progress made in technologies such as gene isolation, promoter identification, gene transfer to monocots or dicots, and tissue specific gene expression, transgenic approaches have surpassed classical and neo-classical plant breeding techniques. From the construction of BAC (bacterial artificial chromosome) libraries (first chickpea BAC library, Rajesh et al. 2004) for map-based isolation of genes to the sequencing of entire genomes of several pulses e.g. pigeon pea (Nagendra et al. 2012; Varshney et al. 2012), chickpea (Varshney et al. 2013), soybean (Huang et al. 2010), bird's-foot trefoil (Sato et al. 2008) and barrel medic or clover (Young et al. 2011), gene identification and isolation is now faster and easier. Advances in molecular biology have brought functional genomics within the reach of common labs, enabling elucidation of gene function using a process called reverse genetics. A major advantage of transgenic technology is that genes from unrelated organisms can be used to develop transgenic plants with new traits. Transgenics using genes from numerous sources have been reported for some pulses (Table 6) such as *Medicago* spp. and chickpea, and have outperformed wild types under stressful temperatures e.g. transgenic chickpea possessing choline oxygenase gene from *Arthrobacter globiformis* accumulated higher levels of GB and hence tolerate low temperature stress (Saradhi and Sharmila 2003). The complexity of tolerance mechanisms to low and high temperatures, which involves several genes and many regulatory pathways, is a major bottleneck in the selection of one or a few genes that provide high levels of tolerance to abiotic stresses in transgenic plants. Another bottleneck is an incomplete understanding of mechanisms in temperature stress tolerance. Further studies to identify the genes related to cold or heat tolerance in food legumes are needed to assist in the development of temperature-tolerant transgenics.

Our findings on genes controlling pollen function in chickpea have revealed that stable sucrose metabolism in anthers is a vital mechanism which affects pollen development during cold stress

in cold-tolerant chickpea genotypes. Investigations on the regulation of expression of differentially-expressed genes in anthers of cold-tolerant genotypes under cold stress indicated that the main categories of genes governing cold tolerance in anthers were carbohydrate/triacylglycerol metabolism, signal transduction, pollen development and transport (Sharma and Nayyar 2014). Most of the genes in these categories were up-regulated. Regulation of gene expression suggests that chickpea anthers use a dual cold tolerance mechanism wherein anthers sustain development under cold by enhancing triacylglycerol and carbohydrate metabolism while pollen grains maintain normal development by regulating pollen development genes (Sharma and Nayyar 2014).

Table 6: List of some temperature stress tolerant transgenic legumes.

TRANSGENIC PLANT	SOURCE	GENE TRANSFERRED	STRESS MITIGATED	REFERENCE
Medicago sativa	*Nicotiana plumbaginifolia*	Mn-SOD cDNA Fe-SOD cDNA	Freezing tolerance Enhanced ROS dismutation	McKersie et al. 1993, 2000
Cicer arietinum	*Arthrobacter globiformis*	cod A (choline oxygenase)	Frost resistance	Pardha Saradhi and Sharmila 2003
Medicago sativa	*Saccharomyces cerevisae*	ScTPS1-ScTPS2	Freezing, heat tolerance	Saurez et al. 2008
Medicago truncatula	*Medicago truncatula*	DREB1C	Freezing tolerance	Chen et al. 2010
Medicago falcata	*Medicago falcata*	MfGolS1	Raffinose accumulation Cold tolerance	Zhao et al. 2012

CONCLUSIONS

Food legumes are sensitive to both high and low-temperature situations. These crops accumulate various biomolecules as a part of their stress defense, but their concentrations usually remain low for a high degree of temperature tolerance. Under such circumstances, protection occurs by exogenous supplementation of protective molecules or by raising temperature-resistant lines via various plant breeding and genetic transformation techniques. There are many instances when transgenics will not meet the expected results when tested under field conditions. Therefore, elaborate studies on the wild germplasm of the target crops and their ecological adaptations are required to gain insight into their performance and stability under field conditions. Similarly,

efforts should be made to understand the mechanisms and possibly the master genes by which some accessions of wild species provide higher levels of tolerance to temperature stress. More consistent and comprehensible lab selection processes involving testing under more pragmatic controlled conditions are also required. This will assist in the formation of a sound basis for protecting leguminous crops from the evident temperature hazards to ensure their availability and improved quality in the future.

Reference:

Mangla Bansal, K. Kukreja, Sunita Suneja and S.S. Dudeja (2014) Symbiotic effectivity of high temperature tolerant mungbean (*Vigna radiata*) rhizobia under different temperature conditions International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7706 Volume 3 Number 12 pp. 807-82