

Understanding Pollen Behaviour under High Temperature for Climate Resilient Breeding

ABSTRACT

Global warming raises challenges for plant reproduction as pollen development and functioning are the most heat-sensitive processes. Hence, it is crucial to understand the mechanisms and processes underlying heat-related male sterility in order to maintain food security. Elevated temperatures elicit acclimation responses that permit pollen development under restricted heat stress conditions; physiological injury leading to pollen development and functioning failure occurs at higher temperature stress. Pollen and the surrounding anther tissues respond to increased temperature at the transcriptome, proteome, and metabolome levels. To counteract the damaging effect of misfolded proteins under heat stress, HSPs (Heat Shock Proteins) accumulate in the cytoplasm and organelles to stabilize, resolubilize, and refold proteins. The pathways leading to the production of carbohydrates, amino acids, phenolic compounds, polyamines, hormones and lipids are interconnected and contribute to the metabolic homeostasis required for the growth and viability of the pollen. The pathways leading to the production of carbohydrates, amino acids, phenolic compounds, polyamines, hormones and lipids are interconnected and contribute to the metabolic homeostasis required for growth and viability of the pollen. Using molecular markers to access specific genomic regions associated with a specific trait along with QTL (Quantitative Trait Loci) fine mapping can identify several candidate genes associated with thermotolerance. A deep understanding of the molecular mechanisms and metabolic processes involved in the stress response to high temperatures in flowers and particularly in the male reproductive organs will be a major step towards developing of effective breeding strategies for high and stable production in crop plants.

Keywords: *Pollen, high temperature, breeding, thermotolerance*

1. INTRODUCTION

"Plants are sessile organisms exposed to changing environments and must adapt to maintain organismal and cellular homeostasis. High or low temperature stresses can be detrimental to all phases of plant development. A clear-cut understanding of the behavioural responses of plants to stress during their reproductive phase is critical to managing agricultural productivity, as most of our food supply is a product of sexual reproduction in flowering plants. The development and functioning of the male gametophyte, or pollen, are among the most temperature-sensitive processes within the plant life cycle" [1,88,89,90]. "Exposure to high temperature episodes often coincides with the reproductive phase of the plant life cycle. As pollen development and functioning are the most heat-sensitive processes that impact plant fertility, it is crucial to understand the mechanisms and processes underlying heat-related male sterility to maintain food security". [35]

2. PLANT REPRODUCTION AT HIGH TEMPERATURE

Reproductive growth reduces production potential significantly in high temperature because it is more susceptible and has a variety of negative impacts, including the loss of buds, flowers, fruits, pods, and seeds [2]. The impact of heat stress on reproductive components during development lowers the harvest index, and the degree and duration of the stress cause different reactions in crops [3]. The development of male and female gametophytes (pollen grain and embryo sac), flower initiation, differentiation of male and female floral parts, micro and megasporogenesis, pollination, micro and megagametogenesis, fertilization, and seed development comprise the reproductive phase. Elevated temperatures cause distinct reactions in each stage, yet, all of these reactions have the same overall impact of decreasing net yield. Heat stress causes problems for both male and female gametophytes. It also inhibits the growth of pollen tubes, reduces ovule function and stigma receptivity, limits embryogenesis, reduces ovule viability, increases ovule abortion, and results in poor seed set [4].

2.1 Pollen development

Sexual reproduction is restricted by the sensitivity of the male gametophyte (pollen) to environmental changes and inadequate conditions [5]. "Within the anther locules, diploid pollen mother cells undergo meiosis to produce a tetrad of four haploid microspores encased in locular fluid, which is then used to make pollen. The liberated microspores expand and divide asymmetrically (pollen mitosis I) to create bigger vegetative and smaller generative cells following their liberation from the tetrad. Then, before pollen is expelled from the anther or during the formation of the pollen tube, the generative cell is engulfed by the vegetative cell and goes through a second mitosis (pollen mitosis II) to produce two sperm cells" [6]. "The tapetum is formed by the innermost layer of the anther wall during the development of the pollen mother cells. This tissue is metabolically active, especially in the early stages of microspore development. It supplies the growing microspores with nutrients, carbohydrates, enzymes, and other substances needed to create exine, or outer pollen wall. The degeneration of the tapetum happens soon after the microspores are released from the tetrad and are closely synchronized with the development of the microspores. Pollen formation depends on the tapetum's proper operation and timely degradation" [7]

3. HEAT-INDUCED POLLEN DEFECTS

Pollen growth is adversely affected by both transient high temperatures and persistently modestly higher day and nighttime temperatures. Meiosis appears when the first heat-induced developmental abnormalities occur. Chromosomal behavior and meiotic cell division may be impacted in addition to increased frequency of crossing over and homologous recombination, resulting in uneven chromosomal separation between spores and the creation of diploid dyads [8]. The abnormal spindle orientation caused the chromosomes to behave as they did during cell division. Microtubules and cytoskeleton dynamics have been examined in vegetative tissues and during pollen tube formation, and it is well known that high temperatures impact these structures. Expanding pollen tubes are more susceptible (35°C, three hours) and damage rises with increasing temperature, requiring temperatures above 40°C in vegetative cells in tobacco or *Arabidopsis* [9]. Chromosome aberration during meiosis appears common in extreme heat stress situations [10].

4. THE IMPACT OF HEAT STRESS ON POLLEN QUALITY

4.1 Pollen Viability

Pollen is one of the most sensitive organs to heat stress. As a result, there may be less fruit set and sterile pollen [10]. Consequently, the temperature at which pollen develops affects crop yield [11]. Numerous species have previously shown how heat stress affects pollen viability, including tomato [12], rice [13], chickpea [14], and soybean [15].

4.2 Pollen Development

Excessive temperatures cause a stop in the early phases of pollen formation. Barley is likewise significantly impacted by heat stress during meiosis [16]. While heat stress at later developmental stages did not effect pollen viability, it reduced fruit set, seed quantity, and pollen viability in bell peppers during microspore mother cell meiosis [17]. Meiosis is a typical sensitive stage for most crops, and pollen's developmental sensitivity to heat stress appears species-specific [18].

4.3 Tapetum

The tapetum is critical to providing metabolites to the pollen, an organ whose growth is highly susceptible to heat stress. For instance, a high temperature of 30°C in barley caused an early meiotic prophase I and an early tapetum breakdown [19]. There have also been reports of common bean tapetum degradation under heat stress. Ten days before anthesis, heat stress in common beans caused structural anomalies in the endoplasmic reticulum pattern of the tapetum, which in turn caused the tapetum to degenerate prematurely [20].

4.4 Opening of Loculi

Heat stress in rice impacted the anther loculi's opening, which decreased pollen fertility [13]. Tomatoes also showed the same outcome. Therefore, preventing the anther loculi from opening could prevent pollen grains from being released [21].

4.5 Pollen Germination

Heat stress can also affect pollen germination, which can stop pollen tubes from growing in addition to pollen development [19]. A decrease in pollen germination was primarily responsible for the drop in tomato fruit sets at increased temperatures [22]. Early pollen development failure may be the cause of reduced germination of pollen under heat stress. Additionally, cotton's in vitro pollen germination decreased in response to high temperatures [23].

5. IMPACT OF HEAT STRESS ON POLLEN METABOLITES

5.1. Carbohydrates

Carbohydrates are essential for plant homeostasis because they provide the energy needed for pollen production and germination, but they also play a part in osmotic balance maintenance, stress signaling, and membrane protection. In

tomato plants, blooms grown at a temperature higher than 32°C displayed decreased pollen viability. These flowers displayed an increase in soluble sugars in the locular fluid but a decrease in soluble sugars in the pollen and anther wall [24]. Soluble sugars collected in the locular fluid result from pollen being modified by high temperatures and decreasing their requirement [25]. A failure in pollen development would likely result from changes in the accumulation of carbohydrates at high temperatures, which would also likely reduce the availability of energy resources and the osmotic power of carbohydrates [26].

5.2. Proline

Proline has been demonstrated to preserve cellular homeostasis, protect membrane integrity, and scavenge reactive oxygen species [27]. By preserving the hydration shells surrounding molecules, proline can stabilize proteins. Proline content in the anthers of heat-tolerant and heat-sensitive cowpea cultivars grown in high temperatures (45°C maximum daily temperature) was analyzed. Heat-sensitive cultivars accumulated the largest quantities of proline in their anthers, although tolerant cultivars' mature pollen had a higher proline abundance than sensitive cultivars' pollen [28].

5.3. Lipids

Membrane fluidity is significantly influenced by lipids, particularly in stressful situations. Bound unsaturated fatty acids became more abundant at high temperatures, but saturated fatty acid quantity fell. The authors hypothesized that an increase in unsaturated fatty acids, which increase membrane fluidity because of the presence of double bonds, may be the source of the damage to membranes produced by high temperatures. The membrane is more susceptible to ROS (Reactive Oxygen Species) assaults because of the unsaturated fatty acid moiety. A change in phospholipid saturation was linked to a decline in pollen viability [15]

5.4 Polyamines

Polyamines can preserve membrane integrity and function as ROS scavengers. Adding spermidine or spermine to the germination medium increased in vitro pollen germination in tomatoes after incubating pollen extract in the media for 20 hours at 33°C [29]. This finding revealed that the amount of polyamines was reduced by heat stress. Spermidine and spermine contents in pollen were decreased after 4 hours of incubation at 38°C, whereas putrescine content rose. These variations were linked to a decline in pollen germination [29].

5.5. Hormones

Steroid hormones called brassinosteroids affect how the body reacts to various abiotic stressors, including heat, salt, and drought. They participate in ROS scavenging and interact with heat shock proteins [30]. The reaction to heat stress also involves ethylene. When plants were treated with ethylene, the amount of pollen that was non-viable under prolonged heat stress (32 °C/26 °C) decreased due to a mutation in the ethylene receptor (nr) [31]. Auxin levels in the anthers of barley and *Arabidopsis* reduced after heat stress (30 °C/25 °C for five days), and which was connected with male sterility [32]. Male sterility was reversed by exogenous auxin treatment. Auxin content was connected with a decrease in pollen viability and germination in the anthers of rice plants that were subjected to 39 °C for 4 hours each day for five days [33]. In hot weather, Abscisic Acid concentrations rose while Gibberellic Acid concentrations fell. Pollen germination was previously negatively correlated with high ABA levels [34].

6. HEAT RESPONSES

6.1 Acclimation

Like other plant cell types, the pollen and the surrounding anther tissues react to a rise in temperature at the transcriptome, proteome, and metabolome levels. It was discovered that these reproductive tissues exhibited several heat-related reactions that have been identified as adaptive in vegetative tissues [35].

6.1.1 Protein Homeostasis in the Cytosol: The Heat Shock Response

"One of the main damaging effects of high temperature results from changes in protein structure. High temperature may interfere with protein function, and if more hydrophobic regions are exposed, proteins can aggregate and become cytotoxic. Failure to prevent the accumulation and aggregation of misfolded proteins may eventually lead to cell death. To counteract these effects, the expression of heat shock protein (HSP) chaperones is induced (at high temperatures) in a process known as the heat shock response (HSR). HSPs accumulate in the cytoplasm and organelles to stabilize, resolubilize, and refold proteins" [36]. "Underlying the HSR is a network of heat stress transcription factors (HSFs) that bind to a palindromic DNA sequence, the heat shock element, to induce the expression of heat-responsive genes" [37]. Proteins of different types of HSPs (i.e. belonging to diverse families, like sHSP, HSP70, HSP90, and HSP100) accumulate in developing anthers and pollen grains after a short period of high-temperature stress [38], which points to a

capacity for both tissues to activate “classical” thermotolerance mechanisms. “One of the main HSFs regulating the HSR is HSFA2, which forms a “superactivator complex” with HSFA1 proteins”[39].

6.1.2 Protein Homeostasis in the ER: The Unfolded Protein Response

A second collection of genes, including those encoding chaperones, is activated in response to high temperatures to shield cells from harmful concentrations of unfolded proteins in the endoplasmic reticulum (ER) [40]. The unfolded protein response (UPR) is a phenomenon where an overabundance of misfolded proteins in the endoplasmic reticulum (ER) results in the increased expression of specific ER protein folding machinery components. Two pathways trigger this response: one relies on the ER membrane releasing bZIP28/bZIP17, and the other is triggered by IRE1 alternately splicing bZIP60 [41]. Several UPR genes are activated by heat, and it has been demonstrated that seedlings of a bZIP28 knockout mutant are heat-sensitive, indicating that the UPR plays a crucial part in the body's overall response to heat stress and thermotolerance. By examining a double knockout mutant of *ire1a* and *ire1b*, which deactivates the RNA-splicing component of the UPR signaling pathway, it was found that the UPR is up-regulated at the transcript and protein levels in male reproductive organs during or soon after a heat shock or prolonged exposure to mild heat, indicating the UPR has a role in acclimating developing pollen to heat [42]. The mutant was found to be fertile at room temperature, but at slightly higher temperatures, it became sterile in males due to decreased viability of mature pollen and changed pollen coat composition [43].

6.1.3 Reactive Oxygen Species Scavenging

The buildup of reactive oxygen species is partially because of the cellular damage that is caused by high temperatures (ROS). ROS are hazardous metabolic byproducts that also function as signaling molecules that control stress reactions [44]. Cells feature substantial ROS scavenging and detoxifying machinery, which comprises enzymes such as catalase, ascorbate peroxidase (APX), and superoxide dismutase, as well as antioxidant compounds including ascorbic acid and flavonoids [45]. Under steady-state conditions, there is a controlled equilibrium between production and scavenging because many genes linked to ROS scavenging are responsive to ROS levels. This equilibrium can be upset by exposure to high temperatures, as heat quickly causes ROS to build up and causes secondary oxidative stress. Heat quickly upregulates the expression of ROS scavengers and antioxidant levels to deal with the excess ROS that arises [46].

6.2 Collapse

6.2.1 Defects related to pollen failure under high temperature

The surrounding tapetal cells and growing pollen have a high sensitivity to heat stress, which frequently causes the tapetal cells to degenerate prematurely and the developing pollen to undergo abnormal developmental or programmed cell death. Several physiological traits of growing pollen may be connected to pollen failure under hot conditions. Primarily, growing pollen and tapetal cells have a large number of mitochondria. Consequently, increased respiration in response to heat stress may produce a disproportionate amount of reactive oxygen species (ROS) that are too large for the protective cellular processes to detoxify adequately, damaging to various cell components. Secondly, diminished transport of carbohydrates and other components required for optimal pollen growth may arise from premature tapetum degeneration or impacts on particular metabolic enzymes under heat stress. When combined, the decreased availability of carbohydrates and the elevated respiration caused by a high number of mitochondria may result in energy reserve depletion and developmental abnormalities. Thirdly, proteins unfold in response to heat. The classical chaperone heat stress response (HSR) usually reduces this effect. Heat causes HSFs (Heat Shock Factors), crucial HSR signaling components, to activate in pollen; yet, the cell cannot establish a complete HSR equivalent to vegetative tissues, which is insufficient to shield and refold proteins. Lastly, it is known that heat stress and reactive oxygen species can affect microtubules and the cytoskeleton. Heat alters the spindle apparatus's orientation during meiotic cell division, resulting in abnormal chromosomal behaviour and the failure of pollen formation [47].

6.2.2 Impact of high-temperature stress on multiple functions of tapetum

Several plant species link stress-induced spore abortion and male sterility to changes in tapetal development and sporadic abnormalities in the endothecium and middle layer, two of the surrounding cell layers [48]. A single layer of endopolyploid cells that encircles the locules of growing microspores and pollen grains makes up the tapetum, an integral component of the male sporangium [49]. The tapetum is metabolically active during normal sporogenesis and is as a nutritive source that gives nearby microspores energy and necessary nutrients [50]. Moreover, the tapetum, a secretory cell layer, supplies cell wall components, such as sporopollenin, for developing the pollen exine layer and enzymes for releasing microspores from the meiotic tetrad [51]. The tapetal cells undergo programmed cell death (PCD) and disintegrate at PMI (Pollen Mitosis I), a later stage of male gametogenesis. For appropriate microspore formation, pollen maturation, and fertility, this controlled tapetum-specific PCD (Programmed Cell Death) and disintegration is necessary [52].

When anthers are subjected to heat stress, they usually exhibit dramatic changes in microspore formation along with an early loss of the tapetal cell layer. These tapetal abnormalities severely impair the development of male gametogenesis

and prevent microspores from completing PMI (Pollen Mitosis I), due to loss of nutritional supply. Severe subcellular changes are seen in the tapetum and the outer three anther wall layers (endothecium, middle layer, and epidermis) including hypertrophy (increased vacuolization), overdevelopment of chloroplasts, and excessive swelling of mitochondria [53]. Furthermore, elevated temperatures significantly suppress transcription, lowering the tapetum's ability and the ability of surrounding anther layers to divide [10].

Growing microspores gather photoassimilates like starch and other carbohydrates and form a potent photosynthetic sink. The carbohydrate reserve is modest during the uninuclear microspore stage, but after PMI, growing spores usually exhibit a fast phase of starch production and rapidly acquire substantial amounts of starch [54]. The amount of starch gradually drops during final pollen maturation as it is broken down into soluble sugars. Over time, accumulated anther sugars provide energy for pollen tube formation, microsporogenesis, and pollen maturation. They also act as an osmolyte, providing pollen resistance to abiotic stresses such as desiccation (sucrose, for example, preserves membrane integrity). Therefore, carbohydrates are crucial role in determining pollen viability and germination capacity and are necessary for pollen growth [55].

6.2.2.1 Sugar metabolism and transport in the tapetal cell layer and microspores

Changes in assimilate supply or modifications in sugar metabolism are the two leading causes of the generally decreased accumulation of starch and other carbohydrate components in abiotically stressed microspores. A lower sugar supply to the reproductive tissues is thought to be the cause of the failure of male spore formation during abiotic stress [56]. Rather than changes in sucrose supply, the changed carbohydrate concentration in stressed anthers is due to changes in sugar metabolism and use. In anthers under abiotic stress, the primary enzymes responsible for the metabolism of sucrose and starch exhibit decreased activity. Differential expression of genes related to sugar cleavage and utilization, sugar transport, and starch synthesis has been observed in heat-stressed microspores of *Sorghum* [57]. More precisely, as has already been noted in heat-stressed tomato anthers (32° C/26 °C Day/night), season-long heat stress (36° C/26 °C Day/night) dramatically lowers the transcript level of cell wall invertase (CWI) in the male gametophyte [58].

The transcriptional down-regulation of the invertase β -d-fructofuranosidase is typically the reason for the absence of starch in abiotically challenged microspores. Invertase enzymes are needed in conjunction with sucrose synthase to break the delivered sugar module. Invertase (INV) is the predominant enzyme that regulates sucrose cleavage in spores and anthers of wheat and several other species [57]. Moreover, extracellular Cell Wall invertases are essential for the unloading of sucrose from the tapetum into the apoplastic space and the creation of a soluble sugar gradient along the various anther fractions because developing microspores are physically isolated from the surrounding tapetal cell layer (no symplastic plasmodesmata channels). Due to the specific down-regulation of CW invertase in anthers under abiotic stress, there are substantial abnormalities in pollen development and viability. This is because it prevents the import of hexose sugar units into growing microspores and hinders the starch buildup in maturing pollen. Stress-induced down-regulation of INV reduces starch accumulation in spores and modifies sucrose consumption in other anther tissues. As a result, it frequently results in the ectopic buildup of sugars in organ types other than microspores, like the endothecium and connective tissue [59]. The morphological abnormalities in anthers under abiotic stress may be attributed to these modifications in partitioning anthersugar [60].

The combinatorial effects of two sugar metabolism pathways, such as reduced sucrose transport and altered sucrose metabolism, result in anther-specific changes in sugar content and the corresponding induction of male sterility under abiotic stress [10].

6.2.3 Starvation hypothesis of tapetal vulnerability to abiotic stress

According to the starvation theory, abiotic stress downregulates of a tapetal cell wall invertase, which lowers the amount of sugars that reach the growing microspores [48]. In vegetative tissues, abiotic stress causes an increase in the synthesis of ABA. The gene(s) encoding tapetal cell wall invertase(s) is downregulated due to the hormone's transportation to the tapetum. There is a decrease in the breakdown of sucrose and a compromise in the supply of hexose to the tapetum and the growing microspores. Reduced Gibberellic Acid levels can affect tapetal growth, delay programmed cell death, and cause tapetal starch mobilization [61].

7. POLLEN AS A SCREENING TOOL FOR HEAT TOLERANCE.

Fruit set and production will be impacted by brief bursts of extreme weather, such as high temperatures, which are expected to happen more frequently in the future climate. As a result, it would benefit plants to demonstrate increased reproductive resilience in the unfavourable temperatures typically encountered during plant reproduction and yield-producing processes like fertilization, embryo development, pollen tube growth, pollen grain development, and germination. Two phases of pollen development (mature microspores at anthesis and microspore mother cell meiosis) are

highly susceptible to high temperatures [17,58]. Reduced pollen germination under high temperatures was the main factor in decreased fertility [62]. Determining how well pollen germination and pollen tube growth function across a broad temperature range will help predict how the plant will grow and set fruit at various temperatures [63].

7.1 Pollen Viability an Index of Abiotic Stresses Tolerance

In many crop species, temperatures over or equivalent to 32 °C reduced pollen viability, pollen retention in the anthers, and pollen germination [64]. The impact of heat stress on pollen viability is linked to changes in the anther's development-related glucose metabolism. The effects of high temperatures have been well shown in several crop types. When wheat florets develop, excessive temperatures might result in total sterility. When pollen mother cells were dividing in the early reproductive phase of a wheat plant, for example, they were subjected to heat stress for three days straight at 32 °C. This caused pollen sterility, which significantly reduced seed laying. Pollen mother cells fail during meiosis when *Sorghum* pollen is killed at temperatures of > 42 °C and 10 °C at night, although this does not affect the survivability of the female pollen [65].

7.1.1 Pollen viability assessment

Numerous techniques can be used to indicate pollen viability. However, many in-vitro germination and pollen viability data have been used to evaluate pollen vitality [66]. Pollen viability is also a prerequisite for high crop output and is crucial to the hybridization process [67]. The vigor and fertility of the pollen can be used to determine its quality. The rate of pollen tube germination and pollen germination speed is referred to as vigor. The proportion and vigor of pollen germination have been measured over time using in vitro assays [68]. Assessment of pollen fertility and germination potential are critical criteria for pollen evaluation, and genotypes can be regarded as good pollinators if their pollen viability is high [69].

Additionally, pollen viability is calculated following the use of chemical hybridizing agents to induce sterility [70]. In the past, several stains, such as pollen viability tests, have been employed to determine the relative estimate of fertilization potential and to evaluate pollen vitality [71]. The assessment of pollen during storage, crop improvement, genetics and fertility research, and crop breeding all depend on the application of trustworthy techniques for determining the functional quality of pollen [72]. Additionally, there is a linear relationship between pollen fertility and germination capacity in some fruit species. Although stain tests were quick and straightforward, pollen germination techniques were occasionally necessary to determine pollen fertility [73].

7.1.2 Methods for improving pollen viability

To increase the number of viable blooms, treat seed-producing plants with a fungicide from the strobilurin group, preferably pyraclostrobin. Various methods exist to administer fungicide, such as directly onto the seeds or into the soil before planting. Alternatively, it could be foliar sprayed following germination [74]. Research aimed at determining the impact of boron on rice reproductive growth, stigma receptivity, pollen viability, and yield qualities revealed that stigma receptivity was reported at 0.4 ppm, and mean pollen vitality was 86% [75]. Moreover, rice plants treated with salicylic acid at 0.1–10 mM under heat stress demonstrated a substantial increase in pollen viability and seed-setting rate compared to the NON-SA treatment [76]. The soil's nitrogen and phosphorus content in *Cucurbita pepo* (Cucurbitaceae) also affects pollen production and performance. The mean total number of staminate flowers per plant was unaffected by nitrogen treatments, however, nitrogen did impact the mean size and number of pollen grains per staminate flower. The commercially suggested pollendosage produced more seeds and fertilized the ovules with faster-growing pollen tubes. The high nitrogen treatment plants developed the pollen. However, phosphorus treatments notably impacted male and female reproductive production. Compared to low-treatment soils, pollen originated from the high phosphorus treatment plants, which produced more seeds and met the commercially recommended dose. Nonetheless, it has been determined that variations in pollen performance can cause heterogeneity in the soil's phosphorus and nitrogen content to impact on the proportion of seeds substantially [77].

8. BREEDING FOR POLLEN THERMO-TOLERANCE

8.1 QTL Mapping for Thermo-Tolerance

Wheat cultivars with markers linked to the length of grain filling during heat stress have already been screened using marker-assisted selection [78]. By classical breeding, thermo-tolerance could also be incorporated into a sensitive genotype via a marker linked to pollen thermo-tolerance. QTL (Quantitative Trait Loci) studies for thermo-tolerance traits have been performed in two ways: (i) by evaluating pollen viability in maize, rice [79], and tomato [80] or (ii) by measuring fruit set in tomato [81] and rice [82]. Breeders can use QTL identification as the basis for more in-depth analyses, such as QTL fine-mapping, which may identify more closely linked molecular markers. Breeders can then use these markers to screen seedlings for thermotolerant genotypes that do not have too much linkage drag. Fine mapping may eventually even result in identifying the essential genes causing thermotolerance. By counting the pods per peduncle in a population of recombinant inbred lines, cowpea QTLs associated with thermo-tolerance have been found [83]. As a result, numerous candidate genes encoding proline transporters, heat shock transcription factors, and heat shock proteins were found [84].

Metabolite profiles, especially in pollen, alter at different temperatures in addition to pollen viability and fruit set. An alternate method of phenotyping pollen thermotolerance is to map the variance in levels of particular metabolites linked to thermo-tolerance. Pollen from genotypes that are thermotolerant retains its sugar levels, while sensitive genotypes see a reduction in sugar levels [85]. It was proposed that sugar level, which is easier to detect and more reliable than pollen viability, would be a **helpful** thermotolerance criterion. Therefore, discovering metabolite QTLs (mQTL) linked to thermotolerance may be a helpful method to gain a fresh understanding of the processes granting thermotolerance [86]. A mapping population's segregation of these metabolites can be used to pinpoint chromosomal areas that may be connected to pollen thermotolerance. The key to identifying thermo-tolerant QTLs and predictive molecular markers is the creation of a **suitable** mapping population and simple, trustworthy phenotyping techniques. **Other methods besides QTL analysis exist** to find potential genes linked to pollen thermotolerance. On the other hand, a thorough understanding of the desired characteristic may identify particular candidate genes whose function in pollen thermotolerance can be examined using reverse genetics techniques (Figure 1) **in which the candidate gene is either overexpressed or downregulated by transgenic or tilling techniques** [84]. **Essential genes** involved in thermo-tolerance may be identified through over-expression or down-regulation of flavonoid pathway genes. These genes might serve as the foundation for discovering (via diversity screens) or producing (through tilling) unique genetic variation that can be utilized as a marker in pollen thermotolerance breeding[84].

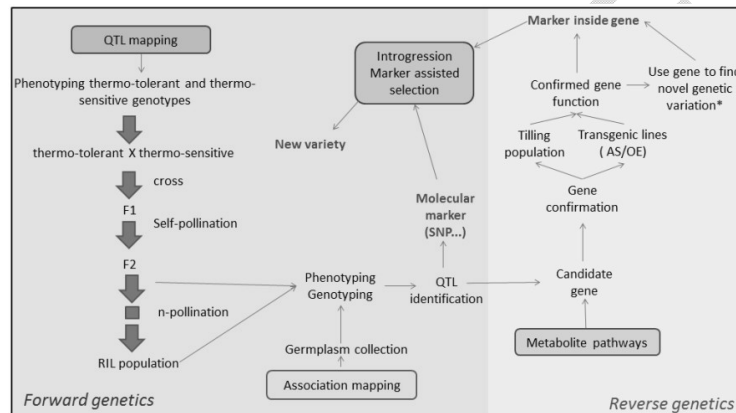


Figure 1: Breeding approaches to improve crop thermo-tolerance

There are two distinct, so-called reverse genetics methods for validating candidate genes. The first involves analyzing the phenotypic of a population that has been tilled (targeted induced local damages in genomes) by focusing on a particular gene. Seeds are mutagenized at random to create a tilling population [87]. The second strategy involves creating transgenic lines where the chosen gene may be either overexpressed or silenced using a constitutive or tissue-specific promoter, for example, or by utilizing an antisense construct. The phenotypic study of these lines might verify that the **candidate gene causes the variation in the population's trait of interest**. For instance, an **over-expression of a potential gene linked to pollen thermo-tolerance** would be expected to result in higher performance at high temperatures than the wild-type[84].

9. CONCLUSION

During the crop growth cycle, the reproductive phase is more susceptible to high temperatures than the vegetative phase. Male reproductive organs are more susceptible to heat stress than female reproductive systems, but the whole reproductive process (from gamete formation to fertilization and seed **maturity**) is very susceptible to high temperatures. Pollen viability, pollen tube development, and decreased stigma **sensitivity prevent** fertilization. Under heat stress, plants respond in **several ways** that provide tolerance, such as by producing signal cascades that significantly alter the expression of specific genes. **Using** "omics" (genomics, transcriptomics, proteomics, and metabolomics) is essential to comprehending the molecular foundations and mechanisms of the plant's heat stress tolerance systems. Because of molecularly linked functional physiology, plants can be developed with increased tolerance to **unfavourable** conditions seen in arid and semi-arid regions of the world, resulting in more profitable yields.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this study are available within the article.

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