Perspective

Pollen Development Under Cold Stress: A Molecular Perspective

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Abstract

Cold stress at reproductive stage induces pollen sterility in susceptible plants. Tolerant plants, on the other hand, develop viable pollen under cold stress. The cause of pollen sterility appears to be the abscisic acid (ABA), the levels of which increase in susceptible plants under cold stress. ABA is down regulator of tapetal cell wall invertases, the enzymes responsible for transport of hexose sugars to the tapetum and microspores. Reduced supply of sugars results in starvation of the developing microspores and consequently the pollen sterility. Molecular mechanisms governing cold tolerance in anthers are poorly understood. The anthers of tolerant plants accumulate low levels of ABA, maintain proper carbohydrate metabolism during microspore development and activate an array of pollen development genes such as pectin methylesterases, β-galactosidases, and transporters of proteins and lipids.

Keywords: Cold tolerance; Cold stress; Pollen; Anther; Transcriptome; Gene expression

Perspective

Flowering followed by pollen development is key to plant reproduction. Pollens develop within anthers where a sporogenous initial cell called pollen mother cell undergoes meiosis to form tetrad. This stage is called microsporogenesis and ends with the release of unicellular microspores from the tetrad by the activity of a mixture of enzymes secreted by the tapetum [1]. The released microspores undergo microgametogenesis via a highly asymmetric division, called Pollen Mitosis I (PM I), to produce a bicellular pollen grain with a small germ cell and a large vegetative cell. The germ cell undergoes a further mitotic division at Pollen Mitosis II (PM II) to produce twin sperm cells. The timing of PM II varies with plant species, sometimes occurring within anthers and mostly during pollen tube growth. The pollen mother cells lie in the locule which is surrounded by a nutritive sporophytic cell layer called tapetum. Tapetum is important for providing nutrients to developing microspores and pollen grains. The pollen development is regulated by gametophytic (microspore/pollen) and sporophytic (tapetum) gene expression [2].

Considerable information has been gained on mechanisms of anther and pollen development in plants [1,3-7]. Two recent developments i.e. whole transcriptome sequencing and laser micro dissection (LM) has considerably added to our knowledge on anther/pollen development. Whole transcriptome sequencing allowed exploration of all genes that express in anthers/pollens. Unlike microarray, no prior knowledge of gene expression is needed for transcriptome sequencing. The use of microarray to study gene expression during anther/pollen development is still being used in crops like Arabidopsis and rice [8,9] where considerable information on genes is available. An anther includes sporophytic as well as gametophytic tissues. While physiological roles of both types of tissues were known, it was not possible to dissect gene expression in gametophytic and sporophytic tissues until the development of LM technique which enabled isolation of specific cells from complicated

tissues [10]. The technique was used to separate transcriptomes of male gametophyte and tapetum in rice and to identify male gametophyte-specific and tapetum-specific genes [11]. Transcriptome exploration based on LM studies has been reviewed and a gene regulation network of anther development in Arabidopsis has been proposed [12]. Similarly, a model of exine precursor biosynthesis in the tapetum cells and transport of the precursors from the tapetum cell to the microspore surface has also been proposed [12]. In addition to genetic control of anther and pollen development, epigenetic reprogramming also seems to play role in microsporogenesis and microgametogenesis[13]. For example, in rice and Arabidopsis, normally silenced transposable elements (TEs) and neighboring sequences, transcribe during meiotic prophase [14,15]. DNA methylation and histone modifications also occur during plant meiosis [16]. To gain an evidence of epigenetic reprogramming during meiosis, 55 genes of putative mitochondrial origin were inserted in a silenced centromeric chromosomal region. These genes showed increased transcription during meiosis [14]. Despite these developments, comprehensive information on molecular mechanisms of anther and pollen development is lacking [17]. For example, the role of phytohormones is far from understood and little is known about the molecular mechanism of formation of the complex exine architecture.

Pollen development is also the target of abiotic stresses such as cold, heat and drought which result in crop yield losses [18,19]. Such stresses may become more important as many agricultural regions may face acute environmental fluctuations in near future owing to climate change [20]. Abiotic stresses can affect the crops at any developmental stage, however, stress at reproductive stage is the weakest link in agricultural productivity [18]. Cold stress at reproductive stage of crops causes pollen sterility with pollen meiosis, the most sensitive stage to cold [21]. The cold also leads to reduction in anther dehiscence, pollen load on the stigma, pollen germination and pollen tube growth [22]. The flowers of cold stressed plants abort and

show reduced yield [21-23]. The anthers show abnormal vacuolation and hypertrophy of the tapetum, unusual starch accumulation in the plastids, premature callose (1,3-beta-glucan) breakdown and lack of normal pollen wall formation [24]. Tapetal development is considered a centre of vulnerability under cold [25,26]. Cold stress delays or inhibits tapetal programmed cell death (PCD), decreases tapetal cell wall invertase levels and amount of hexose sugars reaching the tapetum [26] suggesting that cold interferes with the functioning of the tapetum. Lower levels of sucrose, glucose and fructose result in starvation of developing microspores and consequently lead to pollen sterility [26,27]. It is being believed that ABA is a potential signal for cold-induced pollen sterility [27]. Cold sensitivity in susceptible genotypes is manifested by increase in oxidative stress, increase in membrane damage, decrease in chlorophyll and relative leaf water content [21].

In contrast to cold-susceptible plants, some plants, termed as cold-tolerant, develop viable pollen under cold stress [19]. Such plants produce seeds/grains and yield more than their susceptible counterparts. The cold or other environmental cues are sensed by receptor proteins in plasma membranes. The receptor proteins such as Receptor-like kinases transduce these cues to downstream signalling networks [28] which generate appropriate plant reaction. The receptors act independently or together to initiate downstream signalling events. The stress sensing is followed by ion fluxes which regulate cell osmotic potential, initiate or are part of cellular signal pathways [29] and play crucial role in pollen and pollen tube development [30]. There is no study on perception of cold signals by the anther plasma membranes; however, cold stressed anthers show increased activity of ion transport genes (Sharma and Nayyar, unpublished results). Like cold sensing, the downstream signalling networks operative in anthers under cold tolerance are also poorly understood and information whatsoever available is based on transcriptome studies of anthers under cold stress and normal conditions. Ca^{2+} signalling is involved in cold tolerance or susceptibility in plant tissues [31] including anthers where genes of ca^{2+} signaling pathway were differentially regulated as a result of cold stress ([32], Sharma and Nayyar, unpublished results). In mature pollen of Arabidopsis, down regulation of calcium-binding genes was associated with cold-sensitivity indicating that inhibition of Ca^{2+} signalling was responsible for the cold sensitivity of mature pollen [32]. There are three main families of calcium sensors: calmodulin (CaM), calcineurin B-like (CBL) and Ca^{2+}-dependent protein kinases (CDPKs) in plant systems [31]). Gibberellic acid (GA) also appears to be involved in cold tolerance. In susceptible rice plants, endogenous levels of the bioactive gibberellins GA4 and GA7, and expression levels of the GA biosynthesis genes GA20ox3 and GA3ox1, were less in anthers under low temperature (LT) stress [33]. Rice mutants involved in GA biosynthetic and response pathways were hypersensitive to low temperature stress [33]. Application of exogenous GA significantly reversed the male sterility caused by LT. Simultaneous application of exogenous GA with sucrose further improved the extent of normal pollen development [33]. GA is also required for viable pollen development under normal plant growth conditions [34]. Based on studies on GA-related mutants, it is predicted that the transmission of GA-signalling genes occurred in a sporophytic manner, whereas GA synthesis genes were transmitted in a gametophytic manner [34]. In addition to GA, ABA is also considered a potential signal for

cold-induced pollen sterility [27,35]. In tolerant plants under cold stress, the levels of ABA were low compared to untreated control plants [27,36]. Rice plants transformed with genes that reduce ABA accumulation were fertile and anther ABA levels following cold treatment were significantly lower in these plants [35] indicating that low ABA accumulation is associated with cold tolerance. Auxins and jasmonic acid can be other chemicals associated with cold tolerance/ sensitivity. Genes important for the biosynthesis or signaling of ABA, auxins, and jasmonate, were regulated by cold stress in mature pollen of cold-sensitive Arabidopsis [32]. Maintenance of carbohydrate metabolism, which is vital for viable pollen development in tolerant plants under cold stress, is apparently controlled by ABA. In susceptible plants under abiotic stress, ABA causes reduction in levels of tapetal cell wall invertase [26,27]. The cell wall invertases are involved in apoplectic transport of sugar to the tapetum cells and the developing microspores [37-39] and reduction in their levels lead to hexose sugars starvation of developing microspores [26,27,40]. In addition to their function as nutrition, a category of carbohydrates i.e. beta-galactosidases are associated with pollen expansion after microspore meiosis (Hruba et al. 2005 [41]). Other category of genes associated with pollen development under cold stressis pollen wall development genes such as Pectin methylesterases ([42], Sharma and Nayyar, unpublished results). The PME plays role in tetrad separation and pollen wall synthesis [42] and in Arabidopsis mutated for this gene, pollen grains were released as tetrad [42]. Microsporogenesis followed by pollen development requires continuous supply of wall and other materials. Transcriptome studies have identified genes for intracellular protein transport (SYP124, vesicular mediated transport) and fatty acid transport (peroxisomal ABC transporter [43]) up-regulated in tolerant genotypes under stress (Sharma and Nayyar, unpublished results). These genes play role in pollen maturation, pollen exine formation and pollen tube growth [44].

The information on mechanisms of viable pollen development in anthers of cold tolerant plants is in infancy and primarily focussed on physiological and trancriptomics studies. The biological and molecular functions of several of the transcripts that express differentially in anthers of tolerant plants under stress are still unknown. Moreover, most of the information is from model plants such as Arabidopsis and rice. Bioinformatics tools such as *in silico* promoter analysis can also be exploited to predict cold associated genes. Such analysis has been used to predict cell proliferation associated colon cancer cell lines [45]. The regulation of gene expression in eukaryotes is highly complex and RNA species other than mRNA are also involved in regulating their development. Alternative splicing, transcription start sites and termination sites create heterogeneity in the transcriptional output of multi-exon mammalian and plant loci [46]. At present, nothing is known about the role of such kind of mechanisms in regulating anther/pollen development under cold stress. The epigenetics has received considerable attention in mammalian development. Focus on the role of epigenetics in plant development is a recent phenomenon and epigenetic reprogramming has already been indicted to play role in microsporogeneis and microgametogenesis in plant systems [13]. Such reprogramming cannot be ruled out in cold or abiotic stress tolerance/susceptibility. Understanding molecular mechanisms of pollen development under normal and stressed conditions can also be useful to enhance crop productivity via the development of novel sterile plants that can be used for hybrid seed production in

agriculture. In rice, a new photoperiod-sensitive genic male sterile line applicable for hybrid seed production was developed using a transcription regulator (R2R3 MYB) of pollen development [47].

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