



Corn Crop Production

Growth, Fertilization and Yield

Arn T. Danforth
Editor

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and Policies Series

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CORN CROP PRODUCTION: GROWTH, FERTILIZATION AND YIELD

ARN T. DANFORTH
EDITOR

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Chapter 7

LOW TEMPERATURE EFFECTS ON THE EARLY DEVELOPMENT OF CORN SEEDLINGS

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ABSTRACT

Corn farming is extended all around the world, from temperate regions to tropical ones. Moreover, corn crop represents an essential component of the global food security. In temperate regions, corn growth is reduced by low temperatures at the early stage of development (from germination to fourth leaf fully developed stage). At these stages, corn seedlings are very sensitive to low temperatures as can be determined by several physiological processes. These physiological processes include among others water transport, respiration, photosynthesis and oxygen metabolism. On the other hand, there is a degree on the sensitivity to low temperature among corn genotypes cultivated worldwide. Corn seedlings suffer a decrease in their leaf water content upon exposure to low temperatures. This water deficit is caused by the lack of stomatal response and the diminution of the root water uptake. Together with this water deficit, corn seedlings diminish their respiration and their CO₂ fixation. However, although CO₂ fixation decreases, the amount of light that the leaf receives remains constant. The excess of light non used to fix CO₂ causes an excess of energy in the photosystems, that ultimately is captured by the oxygen molecules, forming the so called reactive oxygen species (ROS). These ROS are highly harmful when they exceed the capacity of the leaves to remove them. Thus the differences on low temperature sensitivity among corn genotypes is partially linked to better water status maintenance, keeping higher rates of photosynthesis, and having more effective ROS removing mechanisms. Here, we will review all these physiological aspects involved on corn seedlings tolerance to low temperatures.

INTRODUCTION

Six to nine thousands years ago teosinte (*Zea mays* ssp. *parviglumis*) was domesticated in Mexico to render the actual corn (*Zea mays* L.) varieties around the world (Yamasaki et al., 2007). Since then, corn crop begun to compromise most of the human food products directly or indirectly like a feed crop (Boomsma and Vyn, 2008). Because corn plants become originally from a tropical region, breeders have been intended to select corn varieties able to growth and yield under colder environmental conditions in order to extend their crop distribution (Enoki et al., 2002; Bhosale et al., 2007; Rodríguez et al., 2008). Thus, there is a degree of cold tolerance among corn genotypes worldwide (Hola et al., 2008; Hund et al., 2008). Corn plants are very sensitive to low temperatures at the early stages of their development, from emergence to fourth leaf stage approximately (Bhosale et al., 2007; Noli et al., 2008). In fact, corn yield increases when seedlings are grown under warm temperatures (Leipner et al., 2008). Corn seedlings sensitive to low temperatures have a strong importance in temperate regions, where corn seeds are sown in the early spring, when occasional low temperatures can still happen, mostly during nights or during early morning or late evening.

Corn seedlings suffer from several physiological disorders when they are exposed to low temperatures. These disorders include among others failure to close the stomata, inhibition of root water uptake, alterations in root and leaf morphology, alterations in respiration quality, decreases of photosynthesis capacity, and increases of oxidative damage (Bos et al., 2000; Ribas-Carbo et al., 2000; Aroca et al., 2003a, 2001b; Hund et al., 2008). In the present chapter I will cover all these physiological disorders, predominately focusing in the differences between cold tolerant and sensitive corn genotypes.

WATER RELATIONS

Cold sensitive plant species including corn retain their stomata opened upon exposure to low temperatures (Irigoyen et al., 1996; Vernieri et al., 2001; Bloom et al., 2004), although some corn tolerant genotypes are able to close them (Capell and Dörffling, 1993; Pérez de Juan et al., 1997; Aroca et al., 2003b). Hence, different sensitivity to cold among corn genotypes is in part related to different capacity of closing stomata under cold conditions (Capell and Dörffling, 1993; Pérez de Juan et al., 1997; Aroca et al., 2003b). By this way, corn tolerant genotypes are able to keep leaf water status at control values during cold periods (Capell and Dörffling, 1993; Pérez de Juan et al., 1997; Aroca et al., 2003b). Since stomatal closure is linked to abscisic acid (ABA) hormone levels in plant tissues (Pei and Kuchitsu, 2005), a correlation between leaf ABA contents and chilling tolerance have also been found (Aroca et al., 2003b; Janowiak et al., 2003). Therefore, cold sensitive corn genotypes decrease its leaf water content upon exposure to cold, because the inability to close their stomata and to increase their ABA contents. However, cold tolerant corn genotypes are able to close their stomata during cold conditions thanks to the increase in their ABA contents, avoiding the water deficit induced by chilling.

In the last few years, it has been well established the mechanisms by which ABA induce stomatal closure (Pei and Kuchitsu, 2005; Wang and Song, 2008). These mechanisms include the generation of nitric oxide and reactive oxygen species, increase of cytosolic calcium,

activation of anion channels, inhibition of inward potassium channels, and activation of potassium efflux. To our knowledge, no studies are available about how these different mechanisms work differently in cold tolerant and cold sensitive corn genotypes. However, there are some studies where the involvement of calcium homeostasis in cold tolerance both in cold sensitive and in tolerant species has been addressed (Song et al., 2008). Thus, Wilkinson et al. (2001) found that *Commelina communis* (cold-tolerant specie) guard cells take up calcium from the apoplast causing stomatal closure upon exposure to 7°C, independently of ABA action, whereas *Nicotiana rustica* (cold-sensitive specie) guard cells did not. On the other hand, Jiang et al. (1999) found a transitory accumulation of calcium in the cytosol and nuclear envelope of winter wheat leaf cells, whereas in maize cells calcium accumulation was permanent upon exposure to 2°C. This different behaviour between the two plant species was caused by a different activity of the Ca²⁺-ATPase, which was inactivated in maize cells.

However, although regulation of stomatal closure under cold conditions seems essential to avoid cold-induced water deficit, in some circumstances, the capacity to keep root water uptake under cold conditions can be crucial (Pavel and Fereres, 1998; Aroca et al., 2001b, 2003b). In 1998, Fennel and Markhart (1998) found an acclimation of root hydraulic properties of spinach plants (cold tolerant specie) after 12 h of exposure to 5°C. Then, although spinach roots initially decrease their root hydraulic conductivity (L), after 12 h at 5°C L increased again (Fennel and Markhart, 1998). Thereafter, Aroca et al. (2001b) carried out a study comparing L behaviour of two corn genotypes differing in chilling sensitivity upon exposure to 5°C. The two genotypes decreased their L during the first hours of cold treatment; however, after 54 h, L of the tolerant genotype was even higher than one of the warm growing plants, whereas L of the sensitive genotype remained at low values. This different behaviour was unrelated to different root ABA contents between the two genotypes (Aroca et al., 2003b).

In the early nineties water transport membrane channel proteins were discovered in plants and named aquaporins (Maurel et al., 1993; Kammerloher et al., 1994). In plants, aquaporins are divided in four groups based on their amino acid sequence homology (Maurel, 2007). The four groups are plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodulin like intrinsic proteins (NIPs), and small and basic intrinsic proteins (SIPs). Based on the evidences supporting the idea that PIP aquaporins could govern L (Kaldenhoff et al., 1998; Javot et al., 2003), Aroca et al. (2005) evaluate the behaviour of different PIPs aquaporins genes and proteins after 3 d of exposure to 5°C in a tolerant and sensitive corn genotypes. Both genotypes increased the amount of PIPs proteins in their roots by cold treatment, but only the tolerant increased its L. At the same time, roots of the sensitive genotype showed symptoms of membrane injury and also its L was very sensitive to exogenous hydrogen peroxide application. These responses to cold were not seen in the tolerant genotype. Therefore, authors concluded that PIPs aquaporins are necessary to recover L from cold, but not sufficient, roots need also to have a strong antioxidant system in order to avoid the oxidative damage linked to cold (Aroca et al., 2005).

In summary, cold corn tolerant genotypes are able to keep their leaf water status at control values upon exposure to cold whereas the sensitive ones suffer from a water deficit. This different behaviour between the two kinds of genotypes is explained at leaf and root levels (Figure 1). At leaf level, cold tolerance is linked to quick stomatal closure and raised ABA contents. At root level, cold tolerance is linked to keep root water uptake rate and to have a strong antioxidant system together with an increase on PIPs aquaporins amount.

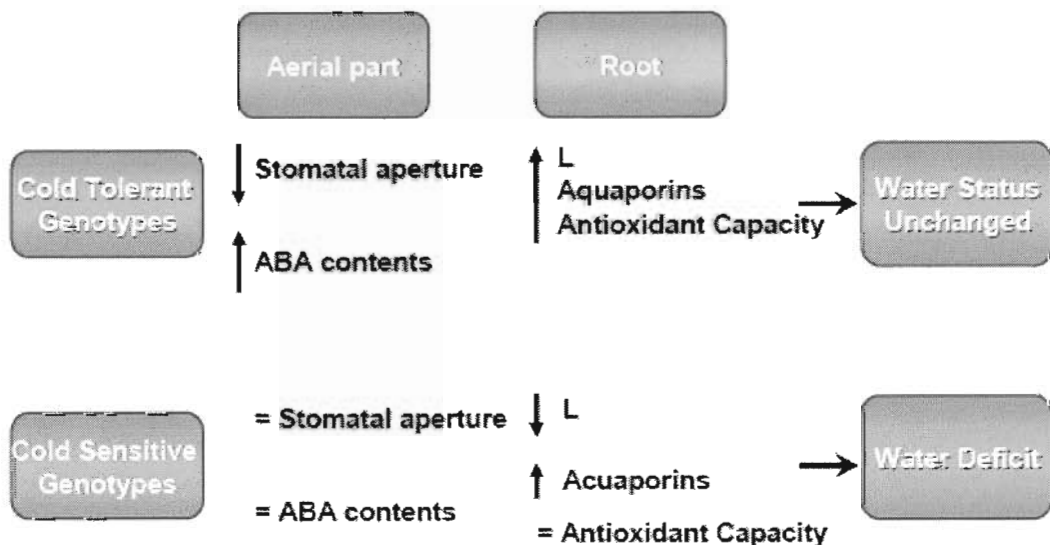


Figure 1. Scheme summarizing different responses to cold of different corn genotypes differing in cold sensitive.

However, which signals are involved in this different behaviour are far to be known, and a comprehensive experiments are needed.

LEAF AND ROOT MORPHOLOGY

At the same time that corn plants suffer from cold-induced water deficit they trait to modify their morphology in order to cope with cold stress. Corn leaf area is determined by five components: leaf-appearance rate, leaf-elongation rate, leaf-elongation duration, maximum leaf width, and leaf shape parameters (Bos et al., 2000). It is well established that low temperatures decrease the rate of leaf appearance (Tollenaar et al., 1979; Thiagarajah and Hunt, 1982), and that different corn hybrids also differ in their appearance rate at low temperatures (Padilla and Otegui, 2005). At the same time, leaf-elongation rate and leaf-elongation duration are decreased and increased by low temperatures, respectively (Bos et al., 2000). However, maximum width is strongly reduced which is associated with a decrease of specific leaf weight (SLW), leading to thinner leaves (Bos et al., 2000). On the other hand, a good correlation between higher SLW and higher leaf photosynthetic efficiency has been observed in different corn hybrids exposed to cold (Hund et al., 2005). Thus, corn hybrids that showed more tolerance to cold, also showed higher SLW and photosynthetic efficiency when grown at low temperatures (Hund et al., 2005).

The growth of corn aerial parts during cold is not only determined by leaf intrinsic properties, but also by root morphology changes under cold conditions (Richner et al., 1997). In fact, a good correlation was found between plant dry weight under cold conditions and chlorophyll content, photosynthetic efficiency and primary lateral root length in different corn hybrids (Hund et al., 2008). As the proportion of root to shoot increases under cold conditions, a better plant performance is found when different corn hybrids are compared (Hund et al., 2008). This pivotal role of root morphology in coping with cold stress could be related in part

to a better nutrient acquisition capacity (Engels and Marschner, 1990; Shabala and Shabala, 2002). However, no information is available about how corns cultivars differing in cold tolerance respond to low temperatures also differ in terms of nutrient acquisition. On the other hand, root morphology can also affects root hydraulic properties. Thus, based on anatomical determinations, Ciamporová and Dekánková (1998) found better root conductivity performance in a cold-tolerant corn genotype than in a sensitive one after exposure to cold.

As summary, differences in leaf and root morphology have been found between cold-tolerant and cold sensitive corn genotypes (Figure 2). The most important differences are related to leaf appearance rate and to SLW, having the tolerant genotypes higher values of these two parameters. At the same time, it is well established that root growth pattern is essential in supporting shoot growth, although the involvement of different root nutrient uptake capacity between cold-tolerant and cold-sensitive corn genotypes need to be addressed.

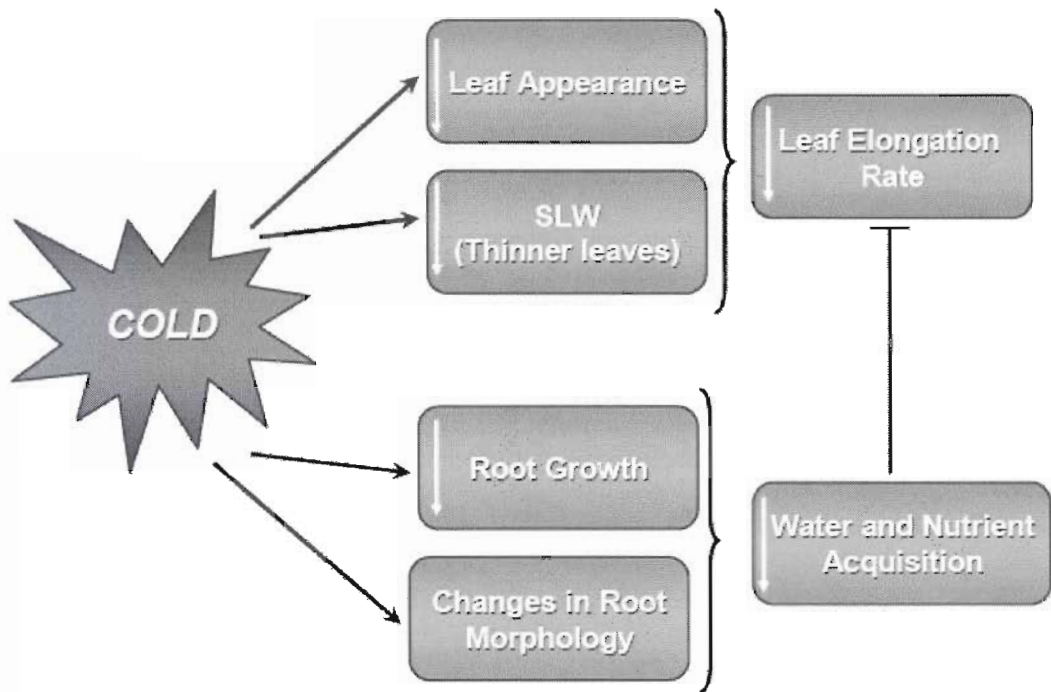


Figure 2. Scheme summarizing cold effects in corn leaf and root morphology.

RESPIRATION

Respiration is needed for several processes in plants like biosynthesis of new structural biomass, translocation of photosynthates from sources to sinks, uptake of ions from soil solution, assimilation of nitrogen and sulphur into organic compounds, protein turnover, and cellular ion-gradient maintenance (Amthor, 2000). Total respiration is divided in growth respiration and maintenance respiration (Amthor, 2000), the later increased under cold

conditions (Frei, 2000). However, although respiration decreases as temperature is going down (Atkin et al., 2000), when plants are growing in cold conditions and respiration measurements are taken under warm conditions, even some increases in total respiration can be observed (Luxová and Gaspáriková, 1999; Atkin et al., 2000; Ribas-Carbo et al., 2000). When different corn genotypes with contrasting cold tolerance were tested in order to see differences in respiration rate, the results varied depending on the organ evaluated (root or leaf). Thus, at leaf level, total respiration rate increased slightly after exposure of corn plants to 5°C during 5 days, independently of the genotype cold-tolerance (Ribas-Carbo et al., 2000). However, Luxová and Gaspáriková (1999), using the same two genotypes as Ribas-carbo et al. (2000), found that total respiration decreased in the sensitive genotype, but it was unaltered in the tolerant one upon exposure to cold.

Respiratory chain electron transport in the inner membrane of the mitochondria in plants can end in two different terminal oxidases, cytochrome oxidase or the alternative oxidase (Hoefnagel et al., 1995). When electron flow ends in the cytochrome oxidase three ATPs are generated, but when electrons ends in the alternative oxidase (AOX) only one ATP is generated (Sluse and Jarmuszkiewicz, 1998). The apparently wasteful of energy by the AOX mitochondrial pathway has focused the attention of several researchers in the last decades (Hoefnagel et al., 1995; Sluse and Jarmuszkiewicz, 1998; Affourtit et al., 2002; Juszczuk and Rychter, 2003). Since AOX dissipates the excess of energy not used to synthesize ATP as heat, it was speculated its involvement in cold tolerance by increasing plant tissues temperature (Moynihan et al., 1995). At the same time, it has been largely observed an increase of the AOX protein amount when several plants are exposed to low temperatures (Vanlerberghe and McIntosh, 1992). However, until 1992 no technique was available to accurately measure AOX activity (Robinson et al., 1992). Such technique is based on the different oxygen discrimination between the two mitochondrial oxidases, avoiding the use of different inhibitors and measuring the real activity of both electron chains (Robinson et al., 1992). Using this technique, Ribas-Carbo et al. (2000) found that both tolerant- and sensitive-cold corn genotypes increased AOX activity after exposure to cold, but the increase was higher in the sensitive genotype. On the other hand, the same authors found that the cytochrome pathway was unaltered in the tolerant genotype but decreased in the sensitive one (Ribas-Carbo et al. 2000). Thus, sensitive genotype was using more respiration activity in a futile way (AOX) than the tolerant genotype. These results pointed out the possibility that AOX activity could be induced by the oxidative stress linked to cold (Foyer et al., 2002), since an increase in the AOX capacity induced by reactive oxygen species had been seen previously (Wagner, 1995). Therefore, AOX activity was found to be related to damage caused by cold, and not to mechanisms involved in cold tolerance (Ribas-Carbo et al., 2000). However, this observation needs to be checked in more corn genotypes in order to clarify AOX role in corn cold tolerance.

PHOTOSYNTHESIS

Plant growth is ultimately determined by the sum of the carbon gained by the photosynthesis and carbon consumed by respiration. In fact, some cold corn sensitive genotypes show a negative growth rate under cold conditions (Irigoyen et al., 1996; Pérez de

Juan et al., 1997; Koscielniak and Biesaga- Koscielniak, 2000). Under cold conditions, photosynthesis is diminished by the means of several factors, being the most important the decrease on carbon assimilation enzymes activity, especially in C_4 plant species like corn (Sage and Kubien, 2007). Corn photosynthesis is divided in two steps, also divided physically in two different kinds of cells, namely mesophyll and bundle cells (Von Caemmerer and Furbank, 2003). In mesophyll cells, anhydride carbonic is fixed by the phosphoenolpyruvate carboxylase (PEPC) as oxalacetate (OAA). The OAA is then transported to the bundle cells in the form of aspartate or malate. Then, both molecules are decarboxyated and render CO_2 , which is then fixed by the Rubisco inside the bundle chloroplasts. The decarboxyated products (pyruvate or phosphoenolpyruvate) are coming back to the mesophyll cells and used again by the PEPC as substrate. For more details about this carbon fixation cycle see Wingler et al. (1999).

Most of these corn enzymes are very sensitive to cold, being the more sensitive and limiting steps of the cycle PEPC and NADP-malate dehydrogenase (NADP-MDH) enzyme activities (Kingston-Smith et al., 1997; Furbank et al., 2000; Aroca et al., 2003a). The sensitive of these two enzyme activities to cold could be caused by a dissociation of the tetrameric form of the enzymes caused by cold (Podestá et al., 1990). Aroca et al. (2003a) found that upon exposure to $5^\circ C$ for 5 days, a cold-tolerant corn genotype showed higher activities of NADP-MDH and PEPC than a cold-sensitive one. At the same time, when both corn genotypes were acclimated to cold by a previous drought treatment, the differences in the activity of these two enzymes were almost insignificant. However, no molecular studies have been carried out to ascertain why these two enzymes of the two genotypes respond differently to cold, e.g. oligomeric studies.

Since carbon fixation is reduced during cold periods, an accumulation of reduced power (NADPH) takes place. This overaccumulation of NADPH in the chloroplasts causes a drastic reduction in the electron flow between photosystem (PS) II and PSI, which ultimately produces NADPH (see Foyer et al., 2002). The most efficient and convenient method to estimate photosystems electron flow is measuring leaf chlorophyll fluorescence emissions (Baker, 2008). Among the chlorophyll fluorescence parameters, the maximum quantum yield of PSII (F_v/F_m) have been used largely to discriminate between cold-tolerance and cold-sensitive maize genotypes (Ribas-Carbo et al., 2000; Aroca et al., 2001a, 2003a; Pimentel et al., 2005). The diminution of electron flow between PSII and PSI causes an excess of energy coming to PSII reaction center (RC) and to light harvest complex (LHC). This excess of energy can be finally captured by oxygen molecules and causes an overproduction of reactive oxygen species (ROS; Foyer et al., 2002). However, leaves have several mechanisms to avoid ROS production by dissipating the excess of energy coming to PSII. These mechanisms include dissociation between PSII RC and LHC (Hong and Xu, 1999) and conversion of energy to heat via xanthophylls cycle (Demmig-Adams and Adams, 2006).

From chlorophyll fluorescence measurements it has been inferred that cold-tolerant corn genotypes are able to dissociate LHC II from PS II RC during cold in order to protect RC from photodamage (Mauro et al., 1997; Aroca et al., 2001a). In fact, Mauro et al. (1997) found different phosphorylation pattern of the LHC II protein complex CP29 in two corn genotypes differing in cold sensitivity. When the conversion of violaxanthin to zeaxanthin was compared between cold-tolerant and cold-sensitive corn genotypes under cold conditions, it was found that sensitive genotypes had more zeaxanthin contents (Haldimann, 1997; Aroca et al., 2003a). Since higher zeaxanthin contents indicate more capacity to dissipate the excedent energy as

heat (Demmig-Adams and Adams, 2006), the above results were interpreted as a sensitive genotypes necessity of more zeaxanthin since they did not have other protective mechanisms.

The capacity of recovering photosynthesis activity from cold stress is as important as the capacity of keeping photosynthetic activity as high as possible during cold (Aroca et al., 2001a; Pietrini et al., 2005). In fact, corn cold-tolerant genotypes recover faster their photosynthetic performance after exposure to cold than sensitive genotypes, and hence they diminish the possibility of producing photooxidative damage (Aroca et al., 2001a).

As summary (Figure 3), the photosynthesis step more likely to be affected by cold is the biochemical fixation of CO_2 . This inhibition on CO_2 fixation causes a diminution on the electron transport between PSII and PSI, causing at the same time the induction of energy dissipation mechanisms in order to avoid possible photooxidative damage. In all these steps corn cold-tolerant genotypes are more resistant than sensitive ones.

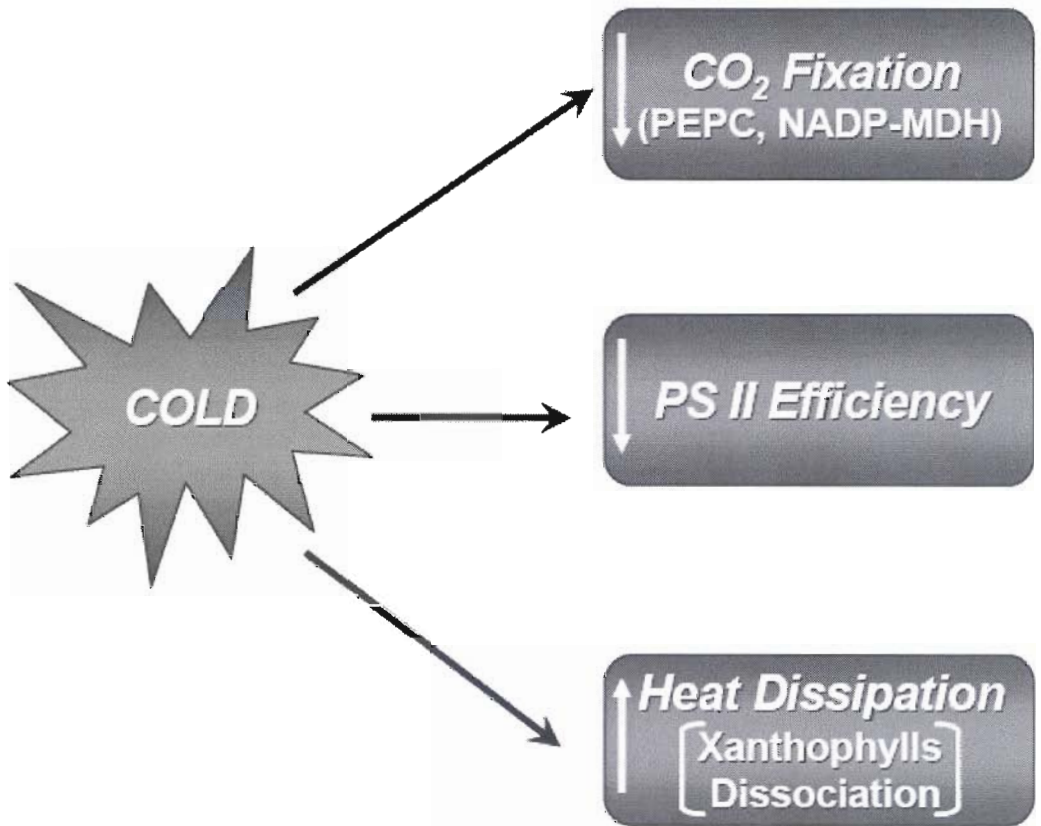


Figure 3. Scheme summarizing cold effects in leaf photosynthesis properties.

ANTIOXIDANT SYSTEMS

As it mentioned in the previous sections, cold injury in sensitive species is ultimately caused by the overproduction of reactive oxygen species (ROS), which can not be detoxified by the plant (Wingsle et al., 1999; Suzuki and Mittler, 2006). Plants have several mechanisms

to detoxify ROS, both enzymatic and non enzymatic antioxidants. The enzymatic antioxidants compromise enzymes from the ascorbate-glutathione cycle (ascorbate peroxidase, APX; glutathione reductase, GR; mono and dehydro ascorbate reductase, MDHAR and DHAR, respectively), superoxide dismutases (SODs), catalases (CAT) and several peroxidases. On the other hand, antioxidant compounds compromise ascorbate (Asc), glutathione (GSH), proline, and several pigments like α -tocopherol or β -carotenoids. For recent reviews on plant antioxidant mechanisms see Ledford and Niyogi (2005) and Navrot et al. (2007).

When different corn genotypes differing in cold sensitivity are exposed to cold, they also differ on the behaviour of antioxidant enzymes, but with contrasting results. Thus, Aroca et al. (2003a) did not find any significant difference in leaf SOD, APX and GR activities between cold-tolerant and cold-sensitive genotypes after plant exposure to 5°C during 5 days. However, Aroca et al. (2001a), studying the same genotypes as in Aroca et al. (2003a), found that at the beginning of cold stress, the tolerant genotype increased about four folds their SOD, APX and GR activities, while not significant changes were observed in the sensitive genotype. These results point out the importance of a faster response of the antioxidants enzymes activity in the tolerant genotype compared to the sensitive one. On the other hand, Leipner et al. (1999) using the same genotypes but grown in the field, found no differences in SOD, APX or CAT activities, but higher GR activity in the tolerant genotype. Hodges et al. (1997) had previously found that tolerant genotypes had more MDHAR activity than sensitive genotypes, pointing out the importance of regenerating ascorbate to properly tolerate cold conditions. All these apparently contradictory results are based on biochemical measurements of antioxidant enzymes activities during different cold treatments. In order to highlight the role of antioxidant enzymes in corn cold tolerance a genetic approach is necessary, that is, getting corn plants with one of the antioxidant enzyme silenced and checking its cold tolerance.

Besides antioxidant enzymes, antioxidant compounds could be even more efficient in avoiding oxidative damage during cold. Hence, Aroca et al. (2003a) found that cold-tolerant genotype had more reduced ascorbate than sensitive one after exposure to cold, although both genotypes had the same amount of total ascorbate. This result indicates the importance of regenerating oxidized ascorbate in order to keep a properly antioxidant system, as previously described (Hodges et al., 1996, 1997). At the same time, when glutathione levels were raised artificially by exogenous application of different herbicides, Kocsy et al. (2001) found an increase also in GR activity, keeping the glutathione pool in a reduced state. Therefore, not only high quantities of ascorbate or glutathione are needed to tolerate cold, but also mechanisms to keep them in a reduced form.

It is well documented that α -tocopherol is an important lipid soluble antioxidant (Li et al., 2008). Leipner et al. (1999) also found that a cold-tolerant corn genotype had more α -tocopherol content in their leaves than a cold-sensitive genotype when they were grown in the field during the spring. Therefore, it is possible that this antioxidant could be also involved on the mechanisms against oxidative damage caused by cold.

Based on what is described above, it is obvious that more efforts are needed in order to understand the specific role of each antioxidant enzyme or compound in corn cold tolerance. Most specifically, genetic approaches are needed where each point of the antioxidant system will be down regulated in order to clarify its function.

CONCLUSION

All the above data support the idea that there are some important differences between cold-tolerant and cold-sensitive corn genotypes. Cold-tolerant genotypes are able to keep their leaf water status almost unchanged under cold conditions, mainly due to their faster stomatal closure and their capacity to keep root water uptake unaltered. At the same time, cold-tolerant genotypes decrease their photosynthesis rate in a less extent than sensitive ones, and also the former have better mechanisms of excess energy dissipation. By contrast, there are still several questions to be addressed in order to clarify the different sensitivity to cold among corn genotypes. (1) How do the different molecular signals involved in stomatal closure behave in the different corn genotypes under cold conditions? (2) Are there any differences in the capacity of root nutrient uptake between the sensitive and tolerant corn genotypes? (3) How do the different antioxidants mechanisms operate under cold conditions in the different corn genotypes? These three questions are some of the open research fields to be studied in the future in order to clarify the different tolerance to cold among corn genotypes.

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