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Clonal response to cold tolerance in creeping bentgrass and role of proline-associated pentose phosphate pathway

Dipayan Sarkar [a], Prasanta C. Bhowmik [a], Young-In Kwon [b], Kalidas Shetty [b,*]

[a] Dept. of Plant, Soil, and Insect Sciences, Stockbridge Hall, University of Massachusetts, Amherst, MA 01003, United States
[b] Dept. of Food Sciences, Chenoweth Laboratory, University of Massachusetts, Amherst, MA 01003, United States

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ABSTRACT

Single seed origin creeping bentgrass ('Penncross') clonal lines were screened to find genetic heterogeneity, which reflected diversity of phenolic production linked to cold stress within a cross-pollinated cultivar. In this study, total soluble phenolic and antioxidant activity varied among 20 creeping bentgrass clonal lines, confirming wide heterogeneity in this cross-pollinated species. Correlations between phenolic content and proline-associated pentose phosphate pathway were also found among the clonal lines. The active metabolic role of proline in cellular metabolic adjustment to cold stress and its support for likely energy synthesis via mitochondrial oxidative phosphorylation was inferred in creeping bentgrass clonal lines based on the activity of proline dehydrogenase. Results of photochemical efficiency of these clonal lines after cold temperature treatment (4 °C) also indicated a close association between stress tolerance and proline-associated pentose phosphate pathway regulation for phenolic biosynthesis and antioxidant response. This study provides a sound metabolic based rationale to screen bentgrass clonal lines for enhanced cold stress tolerance.

1. Introduction

Creeping bentgrass (*Agrostis stolonifera* L.) is a fine textured, stoloniferous turfgrass species used in temperate to warm–humid climate zones for golf course putting greens (Zhang et al., 2002). Turfgrass growing in temperate climates are constantly subjected to varied climatic conditions (Fry and Huang, 2004). Creeping bentgrass is one of the most hardy cool-season turfgrass that can withstand temperature extremes. This ability of creeping bentgrass allows better performance to extreme environmental changes throughout the year. This species has higher ability to tolerate low temperature and maintain good quality turf below freezing, whereas other plant species are not able to sustain their biological function (Turgeon, 1996). Although creeping bentgrass exhibits superior cold hardiness characteristics, winter injury is observed in temperate zones of the United States and in Canada, where winter is severe and prolonged.

Environmental conditions that induce or favor photooxidative stress are common events in growth and developmental processes of plants (Paliyath and Droillard, 1992; Polle, 1997; Thompson et al., 1987) and may have relevance in cold adaptation. As a result of its oxidizing capacity, O_2 acts primarily as an electron acceptor, and leads to the formation of a variety of reactive oxygen intermediates (Asada, 1993). These reactive oxygen species can attack cell membranes by a cascade of free radical chain reactions, resulting in extensive damage to cell membrane and other cellular structures (Halliwell and Gutteridge, 1989). As a part of their aerobic existence, plants have developed an effective defensive system to detoxify reactive intermediates through various antioxidant response mechanisms and this has relevance in cold stress management.

Phenolic compounds are secondary metabolites distributed widely in plants (Javanmardi et al., 2003). The antioxidant activity of phenolics is actually governed by their redox properties, which play crucial roles in absorbing and neutralizing free radicals, quenching single and triplet oxygen or decomposing peroxides (Rice-Evans et al., 1997; Shetty, 2004). In response to biotic and abiotic stress, biosynthesis of plant phenolic antioxidants takes place through the stimulation of secondary metabolite pathways. Few studies have examined the effect of low temperature stress on the levels of phenylpropanoids in plants (Prasad, 1996; Rice-Evans et al., 1997). The recovery from the chilling stress indicates some kind of antioxidant protection; potentially involving anthocyanin and phenylpropanoid biosynthesis (Christie et al., 1994). Direct role of antioxidants in the induction of low temperature tolerance in cool-season turfgrass has not been investigated. However, effects of other oxidative stresses, such as high temperature, low light, ultraviolet-B radiation as well as the role of antioxidative system in stress tolerance of turfgrass have been investigated (Ervin et al., 2004; Jiang et al., 2005; Larkindale and Huang, 2004; Wang

* Corresponding author. Tel.: +1 413 545 1022; fax: +1 413 545 1262.
E-mail address: kalidasshetty@yahoo.com (K. Shetty).

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et al., 2003; Xu and Huang, 2004). In cool-season turfgrass, antioxidant enzymes could either directly enhance low temperature tolerance or induce defensive systems for tolerance through signaling mechanisms. A key regulation of inducible antioxidant enzyme response was suggested to be via proline synthesis (Shetty, 2004).

Amino acids play significant roles in cold hardiness in plants. Accumulation of some specific amino acids during cold acclimation is documented in several plant species (Sagisaka and Araki, 1983; Sakai and Larcher, 1987). The studies conducted on *Poa annua* L. showed higher accumulation of amino acids with cold acclimation (Dionne et al., 2001). After exposure to subfreezing temperature, a higher level of proline, glutamine and glutamic acid in *P. annua* crown were observed (Dionne et al., 2001). Proline is synthesized from glutamate by a series of reduction reactions. In this synthesis process, proline and pyrroline-5-carboxylate (P5C) may regulate redox and hydride ion-mediated stimulation of pentose phosphate pathway (Hagedorn and Phang, 1983; Phang, 1985). During respiration, oxidation reactions produce hydride ions, which help reduction of P5C to proline in the cytosol. Through the reaction of proline dehydrogenase, proline can be oxidized in the mitochondria. Within the mitochondria, instead of NADH, proline can be used as a reducing equivalent and can support oxidative phosphorylation. The reduction of P5C in cytosol provides NADP+, which is a co-factor for glucose-6-phosphate dehydrogenase (G6PDH). G6PDH plays crucial committed role, by catalyzing the first rate limiting step of the pentose phosphate pathway. Phang (1985) first proposed this model and suggested its role in stimulation of purine metabolism via ribose-5-phosphate in animal cells.

On the basis of the Phang (1985) model, Shetty (1997) proposed a model (Fig. 1) that proline-associated pentose phosphate pathway could stimulate both the shikimate and phenylpropanoid pathways, and therefore, the modulation of this pathway could

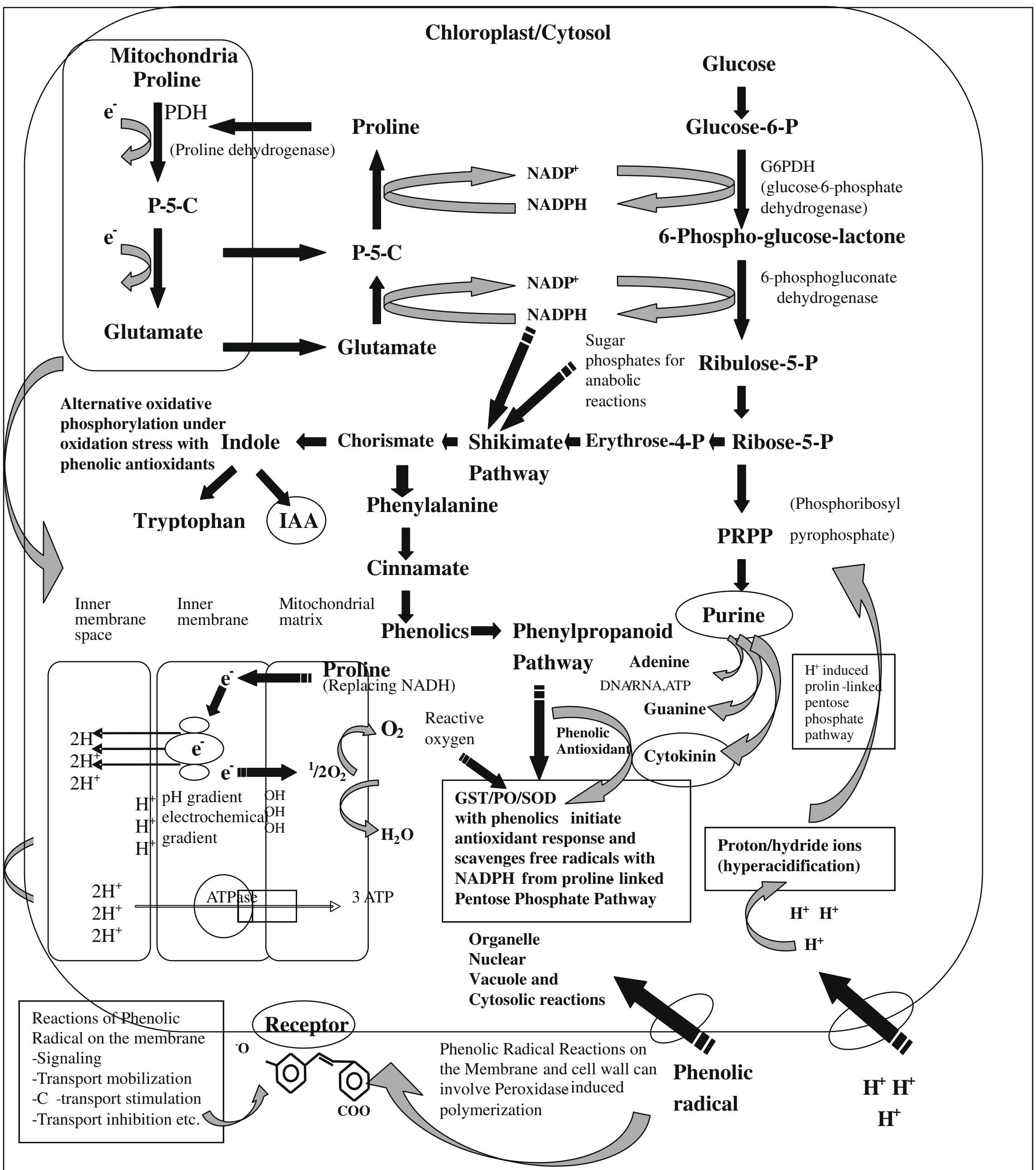

Fig. 1. Model for the role of proline-associated pentose phosphate pathway in regulating phenolic biosynthesis in plants, which also accommodates the mechanism of action of external phenolic phytochemicals.