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Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat

Eliane Cristina Gruszka Vendruscolo[a,*], Ivan Schuster[b], Marcos Pileggi[c], Carlos Alberto Scapim[d], Hugo Bruno Correa Molinari[e], Celso Jamil Marur[e], Luiz Gonzaga Esteves Vieira[e]

[a]*Universidade Federal do Paraná-Campus Palotina, Rua Pioneiro, 2153, CEP 85950-000 Palotina-Pr, Brazil*
[b]*Coodetec-Cooperativa Central de Pesquisa Agrícola, CEP 85813-450 Cascavel-Pr, Brazil*
[c]*Universidade Estadual de Ponta Grossa, Debiogem, CEP 84030-900 Ponta Grossa-Pr, Brazil*
[d]*Universidade Estadual de Maringá, PGM-Agrárias, CEP 87020-900 Maringá-Pr, Brazil*
[e]*Instituto Agronômico do Paraná, CEP 86047-902 Londrina-Pr, Brazil*

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Summary

Water deficit is one of the main abiotic factors that affect spring wheat planted in subtropical regions. Accumulation of proline appears to be a promising approach to maintain the productivity of plants under stress condition. However, morphological alterations and growth reduction are observed in transgenic plants carrying genes coding for osmoprotectants controlled by constitutive promoters. We report here the effects of water deficit on wheat plants transformed with the *Vigna aconitifolia* Δ^1-pyrroline-5-carboxylate synthetase (*P5CS*) cDNA that encodes the key regulatory enzyme in proline biosynthesis, under the control of a stress-induced promoter complex—AIPC. Transgenic wheat plants submitted to 15 days of water shortage presented a distinct response. We have found that drought resulted in the accumulation of proline. The tolerance to water deficit observed in transgenic plants was mainly due to protection mechanisms against oxidative stress and not caused by osmotic adjustment.

Introduction

Water deficit is one of the main abiotic factors affecting spring wheat yields in subtropical latitudes. There has been considerable work in stress-tolerant wheat improvement using classical

Abbreviations: 2,4-D, 2,4-dichlophenoxyacetic acid; AIPC, ABA-inducible promoter complex; PAT, phosphinothricin acetyltransferase; GUS, β-glucuronidase; MDA, malondialdehyde; MPa, megapascal; MS, Murashige and Skoog; MSI, membrane stability index; P5CS, Δ^1-pyrroline-5-carboxylate synthetase; PCR, polymerase chain reaction; PPT, phosphinothricin

*Corresponding author. Tel.: +55 4436493444 R209; fax: +55 4436496616.
E-mail address: vendruscolo@ufpr.br (E.C.G. Vendruscolo).

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breeding. Progress for the development of tolerant genotypes faces some difficulties due to the low heritability of this characteristic, non-uniform testing conditions and high genotype × environment interaction in the selection process (Kirigwi et al., 2004). In this way, plant transformation is an important tool for the development of tolerant genotypes (Sahrawat et al., 2003; Jones, 2005).

Abiotic stress induces the transcription of genes that can be classified into two groups (Shinozaki and Yamaguchi-Shinozaki, 2000). The first group is involved in cellular protection including synthesis of compatible solutes, detoxification of harmful compounds, protein recycling and membrane stabilization (Shinozaki and Yamaguchi-Shinozaki, 1997). The second group includes regulatory genes such as signalling molecules and transcription factors (Seki et al., 2003).

The accumulation of osmolytes during stress is well documented. Recent studies have demonstrated that the manipulation of genes involved in the biosynthesis of low–molecular-weight metabolites, such as proline, have improved plant tolerance to drought and salinity in a number of crops (Molinari et al., 2004; Zhu et al., 2005). Proline has several functions during stress: osmotic adjustment (Voetberg and Sharp, 1991), osmoprotection (Kishor et al., 1995, 2005), free radical scavenger and antioxidant (Sharma and Dietz, 2006), protection of macromolecules from denaturation (Vanrensburg et al., 1993), regulation of cytosolic acidity (Sivakumar et al., 2000) and carbon and nitrogen reserve after stress relief (Díaz et al., 1999).

In plants, as well as in all eukaryotes, *P5CS* gene is present and proline is synthesized from glutamate and ornithine (Adams and Frank, 1980). The glutamate pathway is predominant in plants under water shortage and nitrogen starvation (Delauney and Verma, 1993). Proline is synthesized from glutamate via Δ^1-pyrroline-5-carboxylate (P5C) by two successive reductions, which are catalyzed by P5C synthetase (P5CS) and P5C reductase (P5CR) (Hare et al., 1999). P5CS is a rate-limiting enzyme for the biosynthetic pathway in higher plants, being feedback inhibited by proline (Zhang et al., 1995). In general, there is a consensus that proline has an important role in the adaptation of cells to osmotic stress. However, doubts still persist whether the accumulation of this amino acid provides adaptative advantage or it is only a consequence of changes in the metabolism due to stresses (Serraj and Sinclair, 2002).

Initial attempts to obtain transgenic plants overexpressing proline employed vector constructs with the *P5CS* gene linked to a constitutive promoter, such as CaMV *35S* (Zhang et al., 1995; Sawahel and Hassan, 2002). However, the use of stress-inducible promoters driving the expression of genes coding for the biosynthesis of osmoprotectants seems desirable to avoid unexpected secondary effects that may negatively affect the overall performance of transgenic plants under no-stress conditions (Grover et al., 2001). Also, the presence of transgenes driven by constitutive promoters may result in homology-dependent gene silencing, particularly when the promoter is highly active (Vaucheret et al., 2001). Thus, gene expression under the control of inducible promoters is a preferred strategy to produce plants with transgene-mediated improvements for abiotic stress tolerance.

Using approximately 49 bp of the barley ABA-responsive promoter *hva22* (ABRC–ABA responsive complex) fused to a truncated promoter from the barley α-amilase (Amy 64), coupled with a 1-intron-exon-2 intron-2 of *hva22* gene (*hva22i*), Shen et al. (1996) induced about 30-fold *uidA* gene expression in an experiment with barley aleurone layer suspension cells. One and four copies of the stress-inducible gene expression complex (ABRC) combined with a minimal promoter (*Act1-100P*) and the *hva22i* were tested for β-glucuronidase (GUS) activity (Su et al., 1998). Four copies of ABRC resulted in 50–200% higher GUS activity. However, a single copy of the ABRC element in the stress-inducible promoter driving a *P5CS* cDNA gave higher free proline accumulation and improved the growth of transgenic rice plants when compared to control non-transformed plants.

Zhu et al. (1998) used a 49 bp ABA-responsive element from barley *HVA22* gene fused to a 180 bp rice actin 1 minimal promoter and the *hva22i* element to obtain a stress-inducible promoter (AIPC–ABA-inducible promoter complex). This promoter was used to increase the level of P5CS in transgenic rice plants, which led to a drought- and salt-induced accumulation of the proline content and increased tolerance to both stresses (Zhu et al., 1998; Su and Wu, 2004).

The objective of this work was to evaluate transgenic wheat plants expressing a heterologous *P5CS* gene controlled by the stress-inducible promoter AIPC under water deficit.

Material and methods

Plant transformation and selection of transformants

Wheat plants (*Triticum aestivum* L. cv. CD200126) were grown in a greenhouse with temperatures around