Tolerance to Root Flooding of Wheat Plants (Triticum aestivum L.) Produced with Biotechnological Approaches

Dmitriy V. Tereshonok • Anna Yu. Stepanova • Yulia I. Dolgikh • Ekaterina S. Osipova • Denis V. Belyaev • Boris B. Vartapetian

ABSTRACT
Tolerance to soil flooding of wheat plants produced by in vitro selection of cells more tolerant to anoxia and regeneration of plants from such cells and transgenic wheat plants harboring the ipt gene encoding isopentenyltransferase, a key enzyme of cytokinin synthesis, was studied. On the basis of preliminary data, it was concluded that these biotechnological approaches are promising for improving tolerance to anaerobic stress of plants, which, like wheat, are highly sensitive to hypoxia and anoxia.

Keywords: anoxia, in vitro cell selection, transgenic plants
Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; CaMV, Cauliflower mosaic virus; MS, Murashige and Skoog; NAA, naphthaleneacetic acid

INTRODUCTION
Waterlogged and submerged soils occupy vast territories in many countries (Maltby 1991). Plants inhabiting these lands, especially crops, suffer strongly from such extreme conditions (Musgrave and Ding 1998; Collaku and Harrison 2002). About 20% of areas used for wheat cultivation are subjected to annual flooding (Setter and Waters 2003). Although on waterlogged and submerged soils plants experience the action of a set of unfavorable factors, deficiency of oxygen (hypoxia) and especially its complete absence from the root zone (anoxia) are the main factors resulting in plant damage and even mass plant death (Vartapetian 1978; Kozlovsky 1984; Jackson et al. 1991; Jackson and Black 1993; Visser et al. 2003). Nevertheless, many plant species, predominantly belonging to the wild flora, developed during their evolution properties facilitating to some degree their life not only on waterlogged but even on submerged soils (Armstrong et al. 1994; Jackson and Armstrong 1999; Colmer 2003; Vartapetian et al. 2008). Although some researchers believe that there are more than 20 mechanisms of plant adaptation to anaerobic stress (Setter et al. 2009) recent advances in the studying of causes for damaging and death of sensitive plants under conditions of hypoxia and anoxia and also the mechanisms of tolerant plant adaptation under such extreme conditions confirmed the concept about two main strategies of plant adaptation to anaerobic environment (Vartapetian 1978; Vartapetian and Jackson 1997; Jackson et al. 2009): true plant tolerance realized due to major rearrangement of plant metabolism under hypoxia or even complete oxygen absence (Loreti et al. 2003; Sachs and Vartapetian 2007; Ismail et al. 2009; Magneschi and Perata 2009) and apparent tolerance when plants inhabiting anaerobic medium avoid anaerobiosis due to oxygen transport from aerated plant parts to the organ located in the anaerobic environment (Armstrong et al. 1994; Jackson and Armstrong 1999; Colmer 2003). Available experimental data indicate that true tolerance of plant organs to hypoxia and anoxia is provided due to the control of mobilization and utilization of endogenous carbohydrate sources (Vartapetian and Jackson 1997; Sachs and Vartapetian 2007; Jackson et al. 2009; Magneschi and Perata 2009).

In this work, we studied tolerance to root flooding of wheat plants produced by improved technology of cell selection as compared to that elaborated in experiments with sugarcane (Stepanova et al. 2002). Another approach for improving wheat plant tolerance to root flooding that we also used in this study had a principally other basis. Cytokinin is known to retard plant senescence (Romanov 2009). Since under soil flooding clear signs of senescence and subsequent death are manifested in such sensitive to oxygen deficit plants as wheat (Trought and Drew 1980), we supposed that stimulation of cytokinin synthesis in transgenic wheat plants should delay to some degree their cell senescence and thus counteract the damaging effects of soil flooding. Therefore, the next stage of our work was the investigation of soil flooding tolerance of transgenic wheat plants harboring the ipt gene encoding isopentenyltransferase, a key enzyme of cytokinin synthesis, which have been produced earlier (Stepanova et al. 2006).

MATERIALS AND METHODS
Early-ripening cv. ‘Enita’ of spring soft wheat (Triticum aestivum L.) was used in experiments. Callus was initiated from immature embryos and cultured on the modified Murashige and Skoog (1962) (MS) nutrient medium supplemented with 20 g/l sucrose, 2.5 mg/l 2,4-D, and 10 mg/l AgNO3. The cells were grown at 26 ± 1°C and a 16-h photoperiod (150 μmol photons m−2 s−1). Tolerant callus cells were selected after 32-h anaerobic incubation in modified carbohydrate-free MS medium (Stepanova et al. 2002). For plant regeneration, calluses were transferred to hormone-free MS medium. Plantlets produced were transferred onto half-strength MS and 1 mg/l NAA nutrient medium for rooting. Plants with well-developed roots were planted in soil.

Transgenic wheat plants, which were also used in experiments with root flooding, were produced by Agrobacterium-mediated
Tolerance to root flooding of wheat plants was evaluated. Distinctions were especially evident at higher temperatures. The first group of plants manifested increased tillering, low plant height, and the absence of fertile ears. Differences between transformants and control plants were observed in their morphology and cytokinin levels.

RESULTS

Tolerance to root flooding of wheat plants produced using in vitro cell selection

Plants regenerated from the calli, which had been subjected to oxygen deficiency for 32 h and control plants that did not pass through the selection procedure, were tested under conditions of soil flooding. After 16 days of soil flooding, only 33% of control plants vs. 73% of experimental plants survived.

For elucidation of genetic nature of selected cells and regenerated whole plants, the seeds collected from plants after cell selection were used for generation of progeny and the analysis of descendant tolerance to soil flooding. On the average, 50 seeds were taken from each plant. The progeny of control and transgenic plants were tested under soil flooding at different temperatures: 22 and 32°C. The effect of anaerobic stress was assessed by comparing control and experimental plant survival, growth, and yield.

Data management and statistical analyses were performed using SPSS Statistics 11.0 software. Mean values were expressed with their standard deviations (SD) and compared by ANOVA using Least Square Difference (LSD) test and Student’s T-test at the 0.05 significance level.

DISCUSSION

Our main strategy for obtaining plants tolerant to root flooding was creation of conditions for survival of the cells with improved energy and carbohydrate metabolism, which permitted them to survive oxygen deficiency. Therefore, sucrose was excluded from MS nutrient medium for selection of tolerant cells. Earlier we have demonstrated that in the absence exogenous carbohydrates sugarcane callus cells manifested high sensitivity to anoxia (Stepanova et al. 2006). Application of carbohydrate-free nutrient medium during anaerobic incubation should facilitate selection of the cells with a large store of endogenous carbohydrates or with their more efficient utilization in fermentation, and this permits them to survive oxygen deficiency.
and in the absence of exogenous sources of carbohydrates could be realized due to the regulation of metabolism of endogenous carbohydrate. Tolerance of selected plants had a genetic nature, which was confirmed by inheritance of tolerance to soil flooding in the following seed generation. Thus, in these experiments, we demonstrated for the first time the inheritance of tolerance to soil flooding in plants obtained by in vitro cell selection (Table 1).

Along with in vitro selection of cells tolerant to anaerobic stress that based on earlier demonstration of the key role of energy and carbohydrate metabolism in plant metabolic adaptation to hypoxia and anoxia, we also used a principally other approach for creating plants with improved tolerance to soil flooding, namely genetic engineering, which was earlier repeatedly used for production of plants tolerant to hypoxia and anoxia (Bucher et al. 1994; Tadege et al. 1998; Quimio et al. 2000; Zhang et al. 2000; Rahman et al. 2001; Ellis et al. 2003; Ismond et al. 2003). In most of these studies, to produce tolerant plants, they were transformed with genes of glycolysis and fermentation enzymes, pyruvate decarboxylase and alcohol dehydrogenase (Bucher et al. 1994; Tadege et al. 1998; Quimio et al. 2000; Rahman et al. 2001; Ellis et al. 2003; Ismond et al. 2003). However, because of complex mechanisms of the control of energetic metabolism, the results of these studies were ambiguous. Our experiments with transgenic plants were based on quite different idea. It is known that long-term soil flooding accelerates markedly leaf senescence and even death of such sensitive plants as wheat (Trought and Drew 1980). On the other hand, plant treatment with cytokinins results in a substantial delay of leaf senescence, in particular in the case of stress-induced senescence (Titov et al. 1986). Zhang et al. (2000) demonstrated that insertion of the ipt gene under the SAG12 promoter improved tolerance to anaerobic stress of Arabidopsis plants. Taking into account the results of these studies, we attempted to retard wheat senescence induced by soil flooding by creating transgenic plants with activated cytokinin synthesis. We also took into consideration that, as distinct from arabidopsis, wheat is a monocotyledonous plant of a great economic importance resides and the literature concerning changes in the cytokinin content under oxygen deficit is rather contradictory (Bakhtenko et al. 2007).

We observed marked differences in the effects of 14-day-long soil flooding on wild-type and transgenic plants. The difference in plant yield was especially pronounced: the grain yield of flooded wild-type plants comprised only 2% of control plants, which did not experience flooding, whereas transgenic plant yield was 36% of control (Fig. 3). Keeping in mind that the average seed weight of wild-type and transgenic plants was approximately similar when plants were grown under conditions of normal soil aeration, it becomes evident that their distinct differences manifested under soil flooding indicate that the reason is a beneficial effect of stimulated cytokinin synthesis in transgenic plants. These differences are also evident in the proportion of ears carrying seeds. Thus, in flooded wild-type plants, the proportion of ears with seeds comprised 33%, whereas 90% of transgenic ears had seeds.

In general, our experiments showed that plants obtained by in vitro cell selection and genetic engineering methods were more tolerant to root flooding, and this indicates that approaches used have a considerable promise.

REFERENCES


Colmer TD (2003) Long-distance transport of gases in plants: a perspective on

![Fig. 2 Effect of root flooding on height of wild-type and transgenic wheat plants.](image1)

![Fig. 3 Effect of root flooding on yield of wild-type and transgenic wheat plants.](image2)