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Response of transgenic potato seedlings to allelopathic pressure and the effect of nutrients in the culture medium

Zuo Sheng-Peng [a,b], Li Xiu-Wei [a], Ma Yong-Qing [a,*]

[a] State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, Chinese Academy of Sciences and Department of Water Resource, Yangling 712100, China
[b] College of Environmental Sciences, Anhui Normal University, Wuhu 241000, China

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ABSTRACT

Chinese farmers frequently use a wheat–potato cropping system. The land area planted to transgenic potatoes is increasing because transgenic potatoes have greater resistance to pests and diseases. However, little is known about the bio-compatibility of transgenic potatoes with wheat straw. The objective of this tissue culture study was to determine the allelopathic effects of wheat straw on transgenic potato seedlings. Seedlings were cultured on normal MS medium (normal treatment) and nutrient-deficient MS medium (acclimated treatment) and then transferred to MS medium, which contained wheat straw powder. Wheat straw powder inhibited potato seedling growth in both treatments. Among the parameters analyzed in this study, inhibition was greatest for plant fresh weight and least for plant height. The inhibitive effects of wheat straw were greater for seedling roots compared to shoots. Resistance to allelopathic pressure from wheat straw was greater in acclimated seedlings compared to normal seedlings. This suggested that previous pressure may have induced tolerance in the transgenic potato seedlings. Furthermore, growth inhibition of potato seedlings from the normal treatment increased as the amount of wheat straw powder in the culture medium increased. Calculations indicated that the presence of wheat straw would lead to a 55% reduction in the total biomass of normal potato seedlings compared to a 39% reduction for acclimated seedlings. Parameters such as net photosynthesis rate (Pn) and quantum yield (Y(II)) changed as the nutrient content of the culture medium increased or decreased, but the changes in the parameters were smaller for acclimated seedlings compared to normal seedlings. This suggests that nutrient status during the culture period could help transgenic potato seedlings adapt and compensate for energy loss from seedlings in defending against allelopathic pressure. In summary, the results show that previous exposure to pressures such as nutrient deficiency may increase the allelopathic pressure resistance of transgenic potato seedlings.

1. Introduction

Potato (*Solanum tuberosum* L.), as a significant grain crop and food crop with abundant nutrition, is widely cultivated. Its yield per year is only inferior to wheat, maize and rice, listed as the fourth important crop. However, in recent years, various adverse habitats and poor management of agricultural practices elicited some issues such as degraded quality, tuber malformation, easy germination, hollow heart and pathogens and virus infection during potato growth, which markedly affected final yield and quality of potato [1]. As a result, the technology of plastic film mulching was welcomed by more farmers and applied widely to potato culturing, which obviously enhanced potato yield, improved its quality, induced early maturity and finally increased peasants income. Whereas the plastic film mulching will cause some secondary hazards like rapid decomposition of organic matters, great consumption of soil fertility, the obstacle of seeds emergence, weeds infestation and difficult decomposition of plastic [2]. Therefore, a novel approach named as fallow soil with crop straw mulching was established so as to eliminate and avoid the above-mentioned issues and ensure high yield and perfect quality. In years of field practices of potato production, it was discovered that crop straw covering from maize, wheat, rice and hay grass showed a stimulative effect on potato growth. Among the crop sources, wheat straw mulching for no tillage would acquire a highest yield of potato [3].

In modern agriculture, common potato cultivars exhibited serious reducing quality for natural crossbreed, interspecific hybridization and viral infection, which behaved as low plant, crinkled and curled leaf and decreasing yield with consecutive years. All these will lose potato value for sperm resource [4]. With the development of biological engineering and transgenic techniques, the

* Corresponding author.
E-mail address: mayongqing@ms.iswc.ac.cn (Y.-Q. Ma).

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potato with gene modification will conquer the previous defects and extended gradually to present potato cultivation. A binary vector of plasmids pRO KII/DR containing the divalent ribozyme gene was constructed and transferred into *Agrobacterium tumefaciens* LBA 4404 by triparental conjugation to obtain virus-resistant transgenic potato plants. Zhang et al found that the transgenic potato would inhibit more than 50% of potato leafroll virus (PLRV) and increase over 30% of yield than before, with a highest productivity of 4200 kg/hm^2. Simultaneously, it maintains optimal quality similar to familiar and common potato accessions [5]. Furthermore, more transgenic potato varieties were utilized field practice. Potato (cv. Bintje) was transformed with a gene encoding an oxalate oxidase from wheat under the control of the CaMV35S promoter. Transgenic potato plants produced high constitutive levels of H_2O_2 as visualized by 4-chloro-1-naphthol staining. The resistance of these plants was tested against *Phytophthora infestans* [6]. Potato is a vegetatively propagated crop, which is plagued by numerous disease organisms and pests: Colorado potato beetle (*Leptinotarsa decemlineata* Say), potato virus Y, and potato leaf roll virus to name a few. These particular pests and pathogens are some of the most devastating in North America, with large expense and effort devoted each year to minimizing their effects on potato production. Monsanto has developed, through *A. tumefaciens*-mediated transformation, several genetically modified potato cultivars that provide "built-in" protection from these organisms while maintaining superior agronomic performance [7].

Although transgenic technology in plants, including potato and the like crops, has created superior quality and significant yield of crops, it is informed and paid plenty attentions to relative ecological risks of transplanted genes [8]. Especially, in the west of China, the conventional agriculture like rotation culturing of wheat and potato and/or wheat, maize and potato has displayed some disadvantages such as low resistance to drought and pathogens. So transgenic potato cultivars will be introduced in the future. And then potato accession transplanted by Cu–Zn–SOD and APX genes showed high and comprehensive tolerance to exterior presses from biotic and abiotic factors. In terms of potato as main food resource in western China, it will be a prior choice. However, whether the modified potato will show expected performance and be compatible with other crops, it lacks necessary data, particularly in regard to the relation between wheat residues mulching and modified potato. Accordingly, in the present study, growth response of modified potato with Cu–Zn–SOD and APX genes was investigated under the incorporating wheat straw powder with the solid agar in the tissue culture. During the course, culturing nutrients and wheat quantity were combined to determine wheat straw allelopathy on potato seedling. Genetically modified potato for normal nutrition treatment and its counterpart acclimated to nutrition pressure were adopted as test receptor seedlings. The aims of present research are to (1) analyze photosynthesis effect and fluorescence change of modified potato leaves while facing wheat straw allelopathy, (2) analyze nutrient effects on wheat straw allelopathy and potato resistance to allelopathic press. Hereby, the study findings will reveal interior mechanism of transgenic potato withstanding and adapting environmental stress. Moreover, it also provide valuable information for surveying the coevolution of genetically modified plant and other species in the vicinity as well as exploring sustainable agriculture and safe ecology system.

2. Materials and method

2.1. Wheat planting and sampling

Wheat (cv. No. 22 Xiaoyan) with strong allelopathic potential was cultivated in the field of Institute of Soil and Water conservation, CAS. Six plots were random block designed and partitioned by 3-m-deep concrete block, with 2 m $\times$ 3 m area per one. Field soil was silt clay loam called as Lou soil for more years of tillage, possessing soil fertility for 0.13% of total nitrogen, 120.28 mg kg^{-1} of hydrolyzed nitrogen, 1.29% of organic matters, as well as supplementary fertilizer of 70 kg h m^{-2} for P_2O_5. Generally, soil owns 20.75% of field water capacity and showed 213.35 g kg^{-1} of soil water content before wheat sowing. In wheat growth cycle, local precipitation fluctuates mean rainfall of common years with 250 mm. And no artificial irrigation was involved in wheat development, mainly depending on native weather and field management. Previous tests indicated few organic matters, poor nitrogen and phosphorus but abundant potassium in tested soil. In Guangzhong district of Shaanxi Province, land system is subject chiefly to the rotation cropping between winter wheat and summer maize, which occurred in 70–80% of grain area. The rest will be currently planted by wheat and potato. After these preparations, in the mature period, aerial parts of No. 22 Xiaoyan were cut and left 10 cm length of rudimental stem straw on the ground. Simultaneously, all roots in wheat rhizosphere soil for 0–20 cm depth and the stem residue were collected and washed to clean all impurity. These materials including roots stubbles were dried in room temperature and ground to powder, which were saved in 0–5 °C refrigerator.

2.2. The bioassay of potato seedlings

Transgenic potato (*S. tuberosum*) with CuZnSOD and APX genes [9] was presented by Gyeongsang National University, Korea in 2004 (Gene structure seen in Fig. 1). After in vitro quick propagation of transgenic potato explants for generations, the plantlets were stabilized. Their apical young stems (1.5–2 cm long) were cut and put into solid culture medium 3/2 Murashige and Skoog medium [MS] for further culture and tests. The improved MS, adjusted to pH 5.8, contained 3 ml/L of NAA, 3% sucrose and 0.75% agar, besides plant nutrients. Based on the growth requirement of transgenic potato, they were cultured at 2000 μmol m^{-2} s^{-1} with a photoperiod of 12-h Light and 12-h Dark at 25 ± 1 °C.

The modified potato was cultivated in an improved method of tissue culture based on that of Shibli et al. [10]. The culture medium was designed for four levels such as 1/4MS, 1/2MS, MS and 2MS. And tested transgenic potato seedlings came from two previous treatments of MS and 1/4MS culture bases, which was called normal and acclimated seedlings. The powdered wheat stubble was added into 50-ml culture medium, at 0.1, 0.2, 1 and 2 mg powder (w/v), which correspond to straw mulching of 0.03, 0.06, 0.3 and 0.6 t/ha in the field, respectively. In control plot, no stubble powder was added and was only pure culture medium. Three factors like potato seedling, culture medium and wheat powder quantity were combined in an alternate format. Each treatment was replicated six times. Each replication consisted of three explants of stem tips, also cultured in 200-ml conical flasks. After that, after 30 days culture of transplanted apical stem, the root length, root number per plant, plant height, stem diameter, branch number of

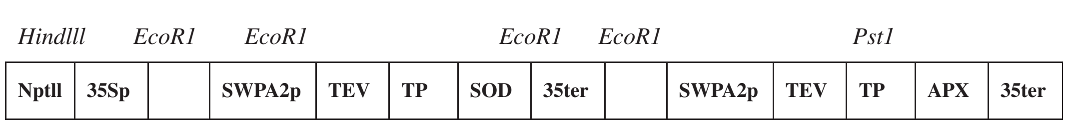

Fig. 1. Schematic SSA for expression vector of CuZnSOD and APX genes transplanted in potato.