Organic potato crops are improved by inoculating a microbial inoculum to the cut surface of seed tubers

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Abstract

Potato seed tubers are usually cut into blocks to reduce seed cost, break dormancy and induce dominance. In chemical farming, the cut surface is usually treated with fungicides to avoid infection. In the present research, the cut surface of the seed tuber blocks was treated with a microbial inoculum (EM) mixed into bamboo charcoal powder and dried for hours. Inoculating and drying the cut trace of seed tuber blocks induced activation of the antioxidant enzymes SOD and POD. Properly drying the cut trace induced osmotic adjustment, leaf turgor improvement, disease resistance and yield increase in the potato crop. The inoculation also induced up-regulation expression of StLTPa1 gene and activation of chitinase and β-1,3-glucanase, which were all responsible for disease resistance. The treatments were more effective in the soil with compost applied onto the surface. The treatments with sterilized inoculum were more effective than that with the original inoculum in improving rooting. In conclusion, inoculating and properly drying cut trace of seed tuber blocks was feasible to improve organic potato crops.

Introduction

Potato seed tubers are usually cut into blocks to reduce seed cost, break dormancy and induce dominance. Usually, cutting into blocks is adopted but drying is usually intentionally avoided (Jenkins et al. 1993, Nielson et al. 1989). However, mildly drying the cut surface might harden the seed tuber and the young plant by inducing xerophytophysiological regulations (Su et al. 2014) and prevent pathogen infections by inducing formation of a cork cell layer (Priestley & Woffenden 1923). In practical, farmers used to paste ash onto the cut surface for prompt drying. Drying the cut trace of the seed tubers is also taken as hardening of the young plants and some positive xerophytophysiological regulation are expected (Xu et al. 2011). In chemical farming, the cut surface of potato seed tubers is also treated with fungicides to avoid infections (Keil et al. 2008). Instead, as one of the nature farming practices, in our experiments the cut surface was inoculated with a microbial material mixed into bamboo charcoal powder and dried mildly. It is reported that *β*-1,3-glucanase and chitinase as key enzymes are responsible for fungal cell and sclerotial wall degradation, as an important factor in biological control (EL-Katatny et al. 2000). It is also reported that *StLTPa1* gene is related with disease resistance in potato plants (Gao et al. 2008). Therefore, activation of these enzymes and expression of the gene were examined in Experiment 1 in this study. In addition, the effects of inoculation and drying the seed tubers were examined in separate experiments in terms of osmotic adjustment, plant growth, photosynthetic activities, disease resistance and the final tuber yield of the organic potato crops.

Material and methods

Experiment 1: Six plants of potato (*Solanum tuberosum* L. cv. Danshaku) were grown in a plastic planter (50 mm × 40 mm × 25 cm). Before planted, the seed tubers were cut each into two pieces and inoculated with either of the followings: 1) CK—bamboo powder only, 2) Original—inoculum (EM as the commercial name, EM Laboratory, Co. Ltd., Shizuoka, Japan) containing lactic bacteria and yeast, 300 time diluted, and 3) Sterilized—heat (120 °C) sterilized inoculum of 2). Activities of chitinase and *β*-1,3-glucanase were analyzed according to El-Katatny et al. (2000). Activities of SOD (superoxide dismutase) and POD (peroxidase) as well as the MDA (malondialdehyde) concentration were measured according to Kakkar et al. (1984). The expression of StLTPa1 gene was analyzed according to Gao et al. (2008).

Experiment 2: With the same potato cultivar as in Exp. 1, experiment was conducted in field conditions with plastic rainout shelters and compost application was taken as the main plot and the cut seed tuber drying as

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the sub-plot in a 2×2 factorial split design. The main plots were arranged in a 2×2 Latin square and one main plot included two sub-plots of 1) cut tubers dried properly, 2) cut tubers not dried. Each sub-plot was arranged in three 12 m × 0.75 m ridges. Space between plants was 0.2 m. Analyses of photosynthesis and osmotic adjustment and photosynthesis were made according to Xu et al. (2011). The data from both experiments were subjected to statistical analysis based on Tukey's multiple comparisons using the software of DPS Data Processing System (Tang and Feng, 2006).

Results

Experiment 1. Chitinase and *β*-1,3-glucanase were activated and as suggested defense mechanisms again pathogen were induced by both types of inoculating (Table 1). Moreover, up-regulation expression of the *StLTPa1* gene was also induced 1 day after inoculation with original inoculum, and as suggested, defense mechanisms against pathogens might be induced because *StLTPs* protein plays several biological roles including antimicrobial defense and signaling. SOD was activated but the inoculations were not stimulations strong enough to largely activate POD. MDA concentration was lower in treatment with both inoculations. It was suggested that the inoculations protected the seed tubers from damage by pathogens. Soluble sugars and protein were in higher concentration in the treatments, which might contribute to improvements in rooting, shooting, and the final tuber yield.

Experiment 2. Leaf turgor potential at full turgid status (P_{FT}) was higher in treatments of drying and also higher in treatment of compost than the controls (Table 2). At the point of incipient plasmolysis, both osmotic potential (π_{IP}) and relative water content (ζ_{IP}) were lower and, as suggested, the desiccation tolerance was higher in treatments of drying and in treatment of compost. The relative water fraction in symplast (ζ_{sym}) was higher in treatments of drying and in treatment of compost than the controls, suggesting that the cell water was recompartmented and part of the water in apoplast moved into the symplast where all biochemical metabolisms occurred. The active net increment of cell solute concentration at full turgid status (ΔC_{FT}) in comparison with the corresponding control was used to show the ability of osmotic adjustment. ΔC_{FT} was higher in treatment of drying. Synergistic interaction between drying the cut surface of seed tubers and applying compost to the soil surface was apparent in all abovementioned variables.

Table 1. Effects of drying cut trace of the seed tubers on tuber yield, disease incidence and photosynthetic activities (Experiment 2).

Inoculation	Chit	Gluc	Gene	SOD	POD	MDA	Proteins	Sugars	Emerg	Yield
Control	0.98_c	47.6_b	0.26B		5.6_C	11.7_b	251.3_a	4.68_b	2.57_b	76.7_b 1372_b
Original	1.28_a	56.6_a	4.93A		28.6_B	29.2_a	172.1_b	5.05_a	3.50_a	83.3_a 1523_a
Sterilized	1.14_b	53.6_a	1.15B		34.8_A	21.0_a	198.1_b	5.11_a	3.93_a	83.5_a 1520_a

Chit, Chitinase activity (Unit g^{-1}FW); Gluc, *β*-1,3-glucanase activity (Unit g^{-1}FW); Gene, relative expression of the non-specific lipid transfer protein gene (*StLTPa1*); SOD, the total activity of superoxide dismutase (Unit g^{-1}FW); POD, activity of peroxidase (Unit g^{-1}FW min^{-1}): MDA, malondialdehyde concentration (mmol kg^{-1}FW); Proteins, concentration of soluble proteins (g kg^{-1} FW); Sugars, concentration of soluble sugars (mmol kg^{-1}FW); Emerg, emergence rate (%); Yield, tuber yield (g pot^{-1}). Lowercase and uppercase letters show difference at p=0.05 and p=0.01.

Table 2. Effects of drying cut trace of the seed tubers on tuber yield, disease incidence and photosynthetic activities (Experiment 2).

Compost	Drying	Yield	DI	L color	P_C	P_{FT}	π_{IP}	ζ_{IP}	ζ_{sym}	ΔC_{FT}
No	No	2.87	21.5	57.4	22.6	0.66	-1.11	0.832	0.74	0.0
	Yes	3.26	14.6	61.7	24.5	0.71	-1.19	0.809	0.68	24.6
Yes	No	3.94	17.3	61.4	26.7	0.69	-1.17	0.831	0.72	8.2
	Yes	4.59	11.2	63.3	27.8	0.78	-1.26	0.819	0.66	49.2
Compost		**	*	**	**	*	*	*	*	**
Drying		**	**	**	*	**	*	**	**	**
Drying×Compost		**	*	*	*	*	*	*	ns	**

Tuber yield (kg m^{-2}); P_C, photosynthetic capacity (μmol m^{-2} s^{-1}); DI, disease index (%); L color. Leaf color (SPAD); P, π, ζ and C mean turgor potential (MPa), osmotic potential (MPa), leaf relative water content and osmotic concentration (osmol m^{-3}), respectively; Subscripts, FT, IP, and sym, mean those at full turgid status, incipient plasmolysis, symplastic water fraction, respectively. * and ** show significance at p=0.05 and p=0.01.

Discussion

Inoculation with the EM microbial materials activated the enzymes of chitinase and the *β*-1,3-glucanase. Chitin is a structural component in organisms including fungi, insects, various crustaceans, and nematode eggs (Cohen 1993). Chitinase hydrolyzes the chitin polymer and plays roles in a defence mechanism in higher plants against attacks by pathogens (Mauch et al. 1988). The *β*-1,3-glucanase is also an enzyme related with pathogenesis and involved in plant resistance against fungi (Boller1985). Non-specific lipid transfer proteins (nsLTPs) in higher plants are lipid binding proteins that play biological roles including antimicrobial signalling and defence against pathogens (Gao et al. 2008). Up-regulation expression of the *StLTPa1* gene was found in the germinating seed potato tubers inoculated with the EM inoculum. SOD were also activated without damages shown by low concentration of MDA by inoculating the microbial materials. The overall results from Experiment 1 in the present research suggested that inoculations to the cut surface of potato seed tubers with the microbial inoculum induced defence mechanisms against the pathogen infections. The objective of Experiment 2 was to confirm whether mildly drying the cut surface of potato seed tubers could induce positive xerophytophysiological regulations in addition to the pathogen defence mechanisms. As shown by the analyses, properly drying cut trace of the seed tuber induced osmotic adjustment and the consequent leaf turgor improvement. Leaf turgor potential is the drying force for cell enlargement in plant growth and for stomatal opening in photosynthesis processes. Another consequence of osmotic adjustment caused by drying seed tubers was cell water recompartmentation between symplast and apoplast, i.e. part of the apoplastic water moved into the symplast, where most biochemical metabolisms occurred (Patakas and Noitsakis 1997). Physiological activities are totally improved and consequently the final tuber yield was increased by the treatment of properly drying cut trace of the seed tubers. The effect in xerophytophysiological regulation was more apparent in potato plants applied with compost into the soil surface layer. In conclusion, organic potato crops could be improved in physiological activities, disease resistance and the final tuber yield by inoculating the EM microbial materials mixed with bamboo powder to the cut surface of the seed tubers that was then mildly dried.

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