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RESEARCH ARTICLE

Improved potato microclonal reproduction with the plant growth-promoting rhizobacteria *Azospirillum*

Oksana V. Tkachenko[1] · Nina V. Evseeva[2] · Natalya V. Boikova[1] · Larisa Yu. Matora[2] · Gennady L. Burygin[2] · Yuriy V. Lobachev[1] · Sergei Yu. Shchyogolev[2]

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Abstract Microclonal propagation in vitro is being actively used in the production of healthy planting material of food and ornamental plants. However, it needs further improvement to increase the growth rates of microclones in vitro and enhance regenerant survivability ex vitro. A promising approach to this end could be inoculating in vitro-micropropagated plants with plant growth-promoting rhizobacteria, specifically *Azospirillum*. However, the influence of *Azospirillum* inoculation on microclone behavior throughout the production process, including plant adaptation ex vitro and food crop productivity, has been underinvestigated. In this study, in vitro-growing potato (*Solanum tuberosum* L.) microclones were inoculated with *Azospirillum brasilense* strain Sp245. The microclones were then grown on in soil in the greenhouse and field, with the experiment lasting for 120 days. Root-associated bacteria were identified immunochemically, and the mitotic index of root meristematic cells was determined by a cytological method. The plant morphological parameters determined were shoot length, number of nodes per shoot, number of roots per plant, maximal root length, leaf area, percentage of surviving plants in the soil, and tuber yield and weight. Our results show that bacterial inoculation of potato microclones in vitro enhances plant adaptive capacity ex vitro and increases minituber yield. The percent survival index of field-grown inoculated plants was 1.5-fold greater than that of uninoculated plants. The overall tuber weight per plant was more than 30 % greater in the inoculated plants than it was in the control ones. For all cultivars on average, tuber yield per square meter increased by more than 45 % as a result of inoculation in vitro. This study is the first to report that *Azospirillum* inoculation of potato microclones not only improves the quality of planting material produced in vitro but also significantly increases minituber yield through enhancing plant adaptive capacity in the field.

Keywords *Solanum tuberosum* L · *Azospirillum brasilense* · Microclonal propagation · In vitro · Ex vitro

✉ Nina V. Evseeva
evseeva@ibppm.sgu.ru

[1] Vavilov Saratov State Agrarian University, 1 Teatralnaya Ploshchad, Saratov 410012, Russian Federation

[2] Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, 13 Prospekt Entuziastov, Saratov 410049, Russian Federation

1 Introduction

Culturing of apical meristems followed by plant microclonal propagation in vitro has been used widely in current biotechnology to produce genetically homogeneous and virus-free planting material of food and ornamental crops (George and Debergh 2008). However, this method needs to be optimized to achieve higher growth rates of microclones and better survival of regenerated plants under soil conditions (ex vitro). The adaptation of test-tube plants to growth in soil is extremely expensive and labor-consuming. Plants that propagate in vitro are sterile, have weak protective systems, and often are difficult to adapt ex vitro, with high percentages of regenerant deaths (Demenko and Lebedev 2011). After being transferred to soil, such plants often become stunted, shed their leaves, and die, primarily because the improper functioning of their stomatal apparatus causes them to lose large amounts of water. Under in vitro conditions, root hairs either do not form or form in numbers insufficient for a normal supply of water and mineral components from soil solution. Therefore, for some species (mostly woody plants or

legumes), a number of symbiological techniques have been utilized, including artificial mycorrhization (Estrada-Luna et al. 2000) and combined treatment with vesicular arbuscular mycorrhizal fungi and *Rhizobium* (Balla et al. 1997). López-Lima et al. (2013) used the nematophagous fungus *Paecilomyces* sp. to efficiently reduce the population of the potato cyst nematode *Globodera rostochiensis*. Work is also ongoing with associative plant growth-promoting rhizobacteria, typically methylobacteria and pseudomonads (Bensalim et al. 1998; Zaharchenko et al. 2012), which can improve the growth characteristics of microclones in vitro and stimulate the acclimatization of regenerants ex vitro.

Plant growth-promoting rhizobacteria supply plants with additional mineral and organic nutrients, phytohormones, and available nitrogen (Vacheron et al. 2013; Bashan et al. 2014; Pii et al. 2015). They also participate in the competitive bioregulation of soil microbial associations (Beneduzi et al. 2012) and induce systemic resistance in the macroorganisms to abiotic and biotic environmental factors (Zamiodis and Pieterse 2012; Paul and Lade 2014). Prominent among them are associative bacteria of the genus *Azospirillum*, which, in particular, can enhance the growth and development of potato microclones in vitro (Volkogon et al. 2006), improve the acclimation of fruit plants ex vitro (Vettori et al. 2010), and increase the productivity of agricultural plants in vivo (Bashan and de-Bashan 2010; Fibach-Paldi et al. 2012). There is, however, a deficit of data to show the influence of bacterial inoculation not only on the growth characteristics of plants cultured in vitro (Volkogon et al. 2006) but also on their adaptation to growth ex vitro.

We hypothesized that developing an active association between in vitro-growing potato (*Solanum tuberosum* L.) microclones and the bacterium *Azospirillum brasilense* Sp245 may not only improve the quality of planting material but also significantly increase minituber yield ex vitro. These improvements would come from the micropartner's positive influence on the resulting plant regenerants throughout acclimatization and from the enhancement of plant adaptive capacity under stressful field conditions. The major aim of the research reported here was to experimentally verify this hypothesis.

2 Materials and methods

2.1 Culturing of potato microclones in vitro

Four potato cultivars were used: Rosara (very early; SaKa Pflanzenzucht GmbH & Co. KG, Germany), Kondor (early-ripening; Agrico, The Netherlands), Nevsky (medium-early; ZAO Vsevolozhskaya selektsionnaya stantsiya, Russian Federation), and Aurora (medium-ripening; ZAO Vsevolozhskaya selektsionnaya stantsiya, Russian Federation). The cultivars were from the plant collection maintained by the Department (*kafedra*) of Crop Breeding, Selection, and Genetics of the Agronomy Faculty at Vavilov State Agrarian University in Saratov, Russian Federation. In vitro-micropropagated plants were separated into microcuttings with one leaf and one lateral bud and were placed in test tubes containing a hormone-free, low-agar (3.5 g L^{-1}) Murashige–Skoog nutrient medium (Murashige and Skoog 1962). The plants were then transferred to a rack and grown for 20 days under the same conditions (temperature, 24 °C; air humidity, 60 %; light intensity, 60 μM m^{-2} s^{-1}; day length, 16 h). Morphological characteristics such as shoot length, number of nodes per shoot, number of roots per plant, and maximal root length were recorded every 5 days.

2.2 Determination of the mitotic index of meristematic cells

On day 20 of plant growth, root-associated bacteria were detected (see Sections 2.5 and 2.6) and the mitotic index of the meristematic cells of adventitious roots from Kondor microclones was determined conventionally (Iordansky 1965). Root tips (2–3 mm) were fixed in acetic acid–ethanol (1:3), stained with acetohematoxylin (Dia-M Co., Moscow, Russian Federation), macerated in cytase (a mixture of enzymes isolated from the stomach of the snail *Helix pomatia*; courtesy of M.I. Tsvetova, Science Research Institute of Agriculture in the South-East, Saratov), and viewed in a Leica DM 2500 microscope (Germany) at ×600 magnification. Each experiment had three replicates, each analyzing root tips excised from five seedlings. In each root tip, not less than 1000 cells were analyzed.

2.3 Inoculation of potato microcuttings

A. brasilense Sp245 (Baldani et al. 1983), a micropartner forming associative relationships with the roots of many plant species, including potato, was obtained from the Collection of Rhizosphere Microorganisms at the Russian Academy of Sciences' Institute of Biochemistry and Physiology of Plants and Microorganisms in Saratov. The culture was grown at 28 °C on a rotary shaker with vigorous agitation (120 rpm) to the end of the exponential phase (18 h) in a liquid malate–salt medium of the following composition (g L^{-1}): Na malate, 5; KH_2PO_4, 0.4; K_2HPO_4, 0.4; NaCl, 0.1; $MgSO_4$, 0.2; $FeSO_4 \cdot 7H_2O$, 0.02; $Na_2MoO_4 \cdot 2H_2O$, 0.002; and NH_4Cl, 1 (pH 6.8–7.0; Döbereiner and Day 1976). The cells were sedimented by centrifugation at 3000×*g* and were suspended in 0.12 M phosphate-buffered saline (pH 7.2) composed as follows (g L^{-1}): KH_2PO_4, 0.43; Na_2HPO_4, 1.68; and NaCl, 7.2. To wash the bacteria free of the culture liquid, we repeated the centrifugation in phosphate-buffered saline twice. Inoculation was performed by our modification of the

procedure of Volkogon et al. (2006). For inoculation, 0.1 mL portions of the resulting bacterial suspension (10^8 cells mL^{-1}) were added to test tubes containing 10 mL of the Murashige–Skoog medium until the final bacterial concentration in the medium was 10^6 cells mL^{-1}. The controls were uninoculated plants grown on a bacteria-free Murashige–Skoog medium.

2.4 Growth of potato plants ex vitro

For adaptation ex vitro, the control and inoculated microplants, after being grown in vitro for 20 days, were transferred to pots of nonsterile soil. The pots were put on a rack in a greenhouse (temperature, 24 °C; air humidity, 60 %; light intensity, 60 μM m^{-2} s^{-1}; day length, 16 h) for 10 days, after which plant morphological characteristics such as height, leaf number, and leaf area were recorded. The plants were then transplanted to a field plot in a checkrow pattern 0.4×0.4 m. The plot is located near the town of Marx in Saratov region, Russia (dark chestnut soil), at a latitude of 51° 36′ 20.5′′ N (51.605694) and a longitude of 46° 31′ 29.72′′ E (46.524922). The weather conditions for field adaptation and growth were not regulated and were stressful for the plants (the daytime air temperature sometimes exceeded 30 °C, the relative humidity was less than 60 %, and the wind speed in a 1-m-high surface layer was greater than 3–5 m s^{-1}). During plant growth, the plot was watered, weeded, supplied with mineral fertilizers, and treated against diseases and pests. Three weeks after planting, as well as at the start of budding and flowering, the percentage of surviving plants, plant height, shoot and leaf numbers per plant, and leaf area were recorded. Tubers were harvested when vines senesced, and the number of tubers per plant, the weight of each tuber, and the overall tuber weight per plant were counted. The plants were grown in the field for 90 days.

2.5 Microbiological test

The viability of *A. brasilense* Sp245 associated with the roots of 20-day-old potato plants was determined by a method modified from that of Zvyagintsev (1991), with account taken of bacterial colonization of root segments. Segments about 5 mm in length were cut from different zones of adventitious roots from the experimental and control plants and were placed on the malate–salt medium (Döbereiner and Day 1976) containing 1.5 % agar. The samples were cultured in a thermostat at 30 °C for 3 days. After that, the bacteria that had grown around the root segments were seeded on the malate–salt medium for immunodiffusion analysis.

2.6 Immunodiffusion

Double immunodiffusion in agarose gel was performed by the standard technique of Ouchterlony and Nilsson (1978). Strain-specific antibodies against *A. brasilense* Sp245 were raised as described by Matora et al. (1998). For bacterial preparations, the cells were washed in phosphate-buffered saline, sedimented by centrifugation, and treated with an extraction buffer (pH 8.5) of 0.1 M Tris–HCl, 10 mM sodium ethylenediaminetetraacetate (EDTA), 0.1 mM phenylmethylsulfonyl fluoride, and 1 % Triton X-100 at room temperature for 30 min. The amount of EDTA was 0.05 mM g^{-1} of wet cells. The extract was freed from the cells by centrifugation, and precipitation was run on 6×9 cm glass plates in 1 % agarose gel prepared with phosphate-buffered saline. The experimental results were evaluated after 18–20 h. The plates were dried, stained with Coomassie Brilliant Blue R-250, and destained in an aqueous solution of 45 % ethanol and 10 % acetic acid.

2.7 Statistics

The experiment was conducted twice (in 2012 and 2013). The experimental and control treatments each had 30 test tubes with plants of each cultivar used. Data from all experiments were processed by two-way ANOVA, which allows evaluation not only of the differences between all treatments but also of the significance of differences for each factor studied (in this case, factor A, reflecting bacterial influence on plant growth and development, and factor B, reflecting the influence of the peculiarities of the genotypes used). The analysis was run with the AGROS program package for statistical and biometrical–genetic analysis in plant breeding and selection (Version 2.09; Department of Statistical Analysis, Russian Academy of Agricultural Sciences). Least significant differences ($LSD_{0.05}$) were determined at a significance level of $p=0.05$. Values followed by different letters differed significantly at $p \leq 0.05$ according to Duncan's multiple range test. In the tables, the letters a, b, c., etc. refer to the sets of data that differed significantly when ANOVA yielded a significant result.

3 Results and discussion

3.1 Bacterial influence on the growth and development of potato microclones in vitro

Our results show that on day 5 of growth, all inoculated microcuttings began to develop one to three rootlets and lateral buds started to grow. None of the test tubes showed opacity around the microcuttings, bacterial films on the surface of the medium, or other signs of contamination.

As found by two-way ANOVA, the inoculated plants of all four cultivars developed more nodes and roots on day 20 of growth than did the control plants (Fig. 1a; Table 1). In addition, the inoculated plants of the early-ripening cultivars Kondor, Rosara, and Nevsky had greater average root lengths

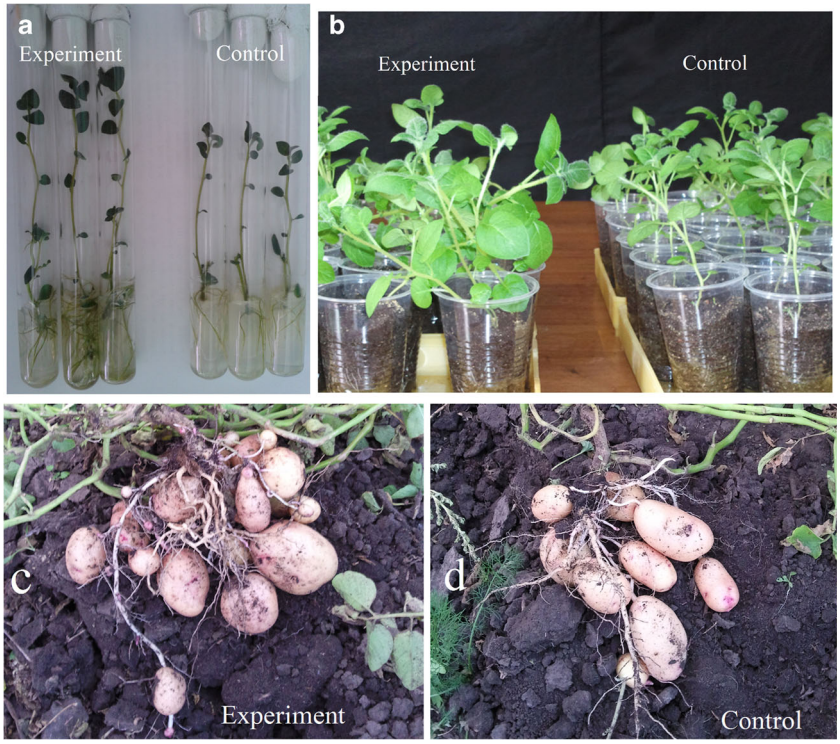

Fig. 1 *Azospirillum* inoculation in vitro during microclonal propagation of potato improves the quality of planting material and increases yield under field conditions (ex vitro). **a** Effect of inoculation with *A. brasilense* Sp245 on the development of in vitro-growing Nevsky microclones. **b** Growth of plants in pot soil ex vitro. The minitubers grown ex vitro from the Nevsky microclones inoculated in vitro with *A. brasilense* Sp245: **c** experiment; **d** control

than did the sterile plants. An exception was the medium-ripening cultivar Aurora, in which the average root length did not differ significantly from that of the controls.

Although there were no significant differences in shoot length between inoculated and control plants, an analysis for factor A (bacterial influence) showed that in all cultivars on average, inoculation had a significant positive effect on all characteristics considered, including shoot length.

Azospirillum inoculation enhanced the mitotic activity of the root meristematic cells of the potato microclones. On day 20 of growth, the mitotic index of the meristematic cells of the inoculated Kondor plants was about 2-fold higher than that for the controls: 2.3 vs. 1.3 % ($LSD_{0.05}$=0.2 %). This finding is consistent with our previous data from a study of wheat seedling inoculation in vivo (Evseeva et al. 2011) and with the data of Levanony and Bashan (1989). It is possible that the mitotic activity enhancement activated the morphogenetic program of development of regenerated plants, which is what we observed in recording the morphological characteristics of the inoculated plants on day 20 of growth (Table 1).

The microbiological test showed that in the experimental treatments, the root segments were covered with bacteria on day 3 of growth in petri dishes containing MSM (Fig. 2a). The bacteria were identified as *A. brasilense* Sp245 by an immunochemical assay with strain-specific antibodies (Fig. 2b).

Our present data agree with those of Volkogon et al. (2006), who found that *Azospirillum* inoculation of in vitro-growing potato microclones increased the intensity of shoot (1.5–2-fold) and root (2–2.5-fold) development. From our data, the growth rates of the inoculated plants also increased as compared to the control values, and the inoculated plants had a better developed root system with many points of growth (Table 1). However, this work differs from Volkogon et al.'s (2006) in that the influence of azospirilla on the growth of various potato genotypes in vitro has been examined in a broader view and the adaptation of these plants ex vitro has been assessed for the first time.

3.2 Bacterial influence on the adaptation of potato microclones ex vitro

As a result of bacterial inoculation in vitro, the next stage (growth in soil) replaced sterile plants with an actively functioning, stable associative plant–microbe system. When the plants were transferred to soil in the greenhouse, favorable conditions were generated that reduced the stress of acclimatization. Because of this, all plants regained turgor within a day after being moved to soil. On day 3, signs emerged that new leaves were being formed. The plant survival index was 100 %, so we were unable to judge the effect of bacteria at this stage. However, after 10 days of acclimatization, some differences were noted between inoculated and sterile plants. Specifically, the inoculated plants exhibited statistically significant increases in node and leaf numbers (Aurora and Rosara), a slight increase in shoot length (Condor), and an increase in leaf area (Nevsky), as compared to the controls (Fig. 1b, Table 2). In all cultivars on average, the

Table 1 Effect of bacterial inoculation on the growth of microclones in vitro and on their adaptation to growth in soil pots (ex vitro)

Cultivar	Treatment	Microclone parameters in vitro				Parameters of the microclones grown in soil pots		
		Node number (pcs)	Shoot length (mm)	Root number (pcs)	Root length (mm)	Node number (pcs)	Shoot length (mm)	Leaf area per plant (dm^2)
Kondor	Control	4.76a	38.5	5.16a	48.3 cd	11.0cde	96.0b	0.0692ab
	Experiment	6.18cd	55.9	7.25cde	54.1e	12.3e	142e	0.0731ab
Aurora	Control	6.13bcd	44.0	5.03a	37.3a	11.9de	68.7a	0.0596a
	Experiment	7.48f	52.9	7.33de	41.8a	15.0f	75.3a	0.0732ab
Nevsky	Control	4.91a	48.6	4.8a	41.8a	7.31a	59.9a	0.0903b
	Experiment	6.72de	54.4	6.52bcde	47.3bcd	8.42ab	64.6a	0.1220c
Rosara	Control	4.80a	67.0	4.92a	41.9a	7.25a	133de	0.0697ab
	Experiment	7.32ef	72.9	7.37e	48.4d	8.70bc	135cde	0.0983bc
For all cultivars on average	Control	5.94a	47.8a	5.90a	44.9a	9.13a	89.8a	0.0722a
	Experiment	6.14b	60.7b	6.20b	45.4b	11.4b	104b	0.0916b

establishment of a plant–rhizobacterial association (factor A) significantly improved the rate of shoot growth through an increased shoot length, the formation of new nodes with leaves, and an increased leaf surface area.

After 10 days of growth in the greenhouse, the in vitro-micropropagated plants not only regained stem and leaf turgor but also formed two to three new leaves. This indicated that the ex vitro acclimatization period had ended and that the potato plants inoculated at the stage of microclonal propagation in vitro were quite successful in tolerating the transfer to soil under controlled greenhouse conditions.

Zaharchenko et al. (2012) reported that methylobacteria had a significant positive effect on growth of the above- and belowground portions of tobacco, rape, and cabbage when micropropagated plants were moved to pots of soil after 8 weeks of growth in vitro. In our experiment, the adaptation took just 10 days, possibly accounting for the lack of a substantial influence of bacteria on plant growth.

3.3 Bacterial influence on the field growth of in vitro-micropropagated plants and on the yield of tubers

The plants were then transplanted to a field plot. Under the uncontrolled stress conditions, 75 to 88 % of the plants died. However, the inoculated plants tolerated the stress much better than did their sterile counterparts. For all cultivars individually and on average, the percent survival index of the inoculated plants was 1.5-fold greater than that of the sterile plants (Fig. 3a).

Plants that had adapted to growing in the field started to form new leaves and stems. At the start of budding and flowering, after the vegetative mass had formed, plant leaf area was measured and found to be significantly greater in the inoculated plants than in the control ones. This fact was established for all cultivars individually and on average.

When vines senesced, the number of tubers and the overall tuber weight per plant were counted. The data showed that for all cultivars except Aurora, the numbers of tubers formed by the experimental and control plants were not significantly different and were dependent to a greater extent on the peculiarities of the cultivar used. However, an analysis for factor A (bacterial influence) showed that in all cultivars on average, inoculation increased the number of tubers per plant (Table 2). In all cultivars individually (except the very early cultivar Rosara) and on average, the overall tuber weight per plant was more than 30 % greater in the inoculated plants than it was in the control ones. On a per square meter basis, all cultivars taken together and each one individually exhibited significantly higher tuber yields (by more than 45 % on average) as a result of having been inoculated in vitro (Figs. 1c, d and 3b).

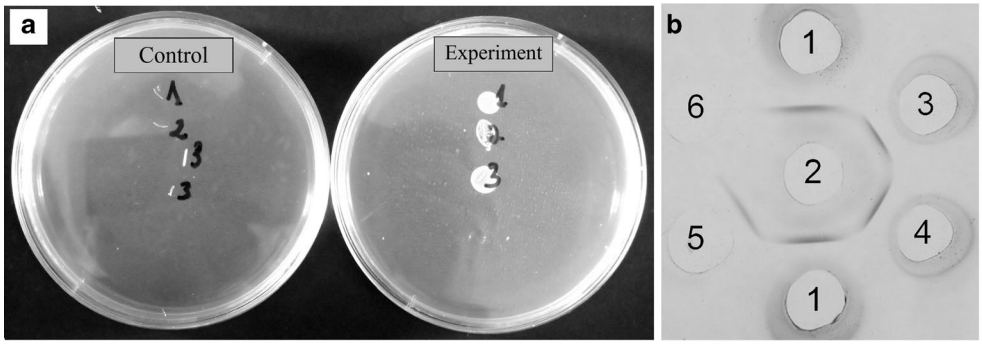

Fig. 2 Detection of *A. brasilense* Sp245 in the microbiological test (**a**) and in the immunochemical assay system (**b**) for the Kondor potato cultivar. A: *1*, *2*, *3*, root segments from 20-day-old potato microclones grown in vitro; B: *1*, *2*, *3*, extracts from the bacteria that had grown around the corresponding root segments; *4*, extract from *A. brasilense* Sp245 cells (positive control); *5*, antibodies; *6*, phosphate-buffered saline (negative control)

Several authors have also reported increased potato yields from tubers treated with plant growth-promoting rhizobacteria, in particular pseudomonads, before planting (Grosch et al. 2005; Rosyidah et al. 2013; Vacheron et al. 2013). Besides promoting plant growth, some strains of *Bacillus*, *Methylobacter*, *Pseudomonas, Azospirillum*, and combined treatment with vesicular arbuscular mycorrhizal fungi and *Rhizobium* influence stress resistance in plants and crop yields (Bensalim et al. 1998; Dey et al. 2004; Saleh and Saleh 2006; Maksimov et al. 2011; Zaharchenko et al. 2012; Pii et al. 2015). *A. brasilense* Sp245 increases the resistance of wheat to osmotic stress by improving the plant's ability to absorb water and nutrients (Creus et al. 2004) and promotes the formation of root hairs in tomato (Creus et al. 2005).

At this point in our research, we cannot suggest any concrete mechanisms that might be responsible for the effect of *Azospirillum* on the growth and development of potato microclones. Azospirilla are known to be effective N_2 fixers, P mobilizers, phytohormone producers, and biocontrol agents (Bashan and de-Bashan 2010; Venieraki et al. 2011; Fibach-Paldi et al. 2012). The use of azospirilla for inoculation at the early stages of microclonal propagation, as against other plant growth-promoting rhizobacteria, was dictated by the greater concentration of bacteria in the rhizosphere. In our experiments, this fact was vividly confirmed by the absence of bacterial overgrowth on the culture medium in vitro (Fig. 1a). We speculate also that in addition to promoting plant growth, azospirilla, like methylobacteria and pseudomonads, can induce systemic resistance to stress in regenerated potato plants. An indication of this can be found in a study by Tortora et al. (2012). In our experiments, the inoculated plants began vegetative growth quicker (at the cost of their enhanced adaptability) and formed more leaves with a larger surface area (Table 2). The better developed assimilating leaf surface enabled the plants to synthesize greater quantities of organic substances. Although the bacteria had little effect on tuber initiation, the overall tuber weight in the inoculated plants was much greater than that in the control ones (Table 2). Consequently, the increased adaptability of the plants in association with *Azospirillum* improved not only plant survival but also tuber yield (Fig. 3).

The results of our study also demonstrate that bacterial inoculation of plants in vitro has a positive effect on the development of the root system. This seems to occur through the synthesis of phytohormones and through the enhancement of the functional activity of root meristematic cells (Bashan and

Table 2 Effect of bacterial inoculation in vitro on the growth of microclones in the field (ex vitro)

Cultivar	Treatment	Leaf area per plant (dm^2)	Number of tubers per plant (pcs)	Overall tuber weight per plant (kg)
Kondor	Control	5.99a	7.75a	0.315ab
	Experiment	15.2b	10.5ab	0.522 cd
Aurora	Control	6.78a	11.1ab	0.281a
	Experiment	14.5b	17.8d	0.567 cd
Nevsky	Control	7.96a	6.25a	0.288a
	Experiment	26.0b	9.75ab	0.415b
Rosara	Control	6.88a	14.6c	0.727d
	Experiment	10.3b	16.4 cd	0.764d
For all cultivars on average	Control	6.90a	10.4a	0.353a
	Experiment	16.5b	13.1b	0.564b

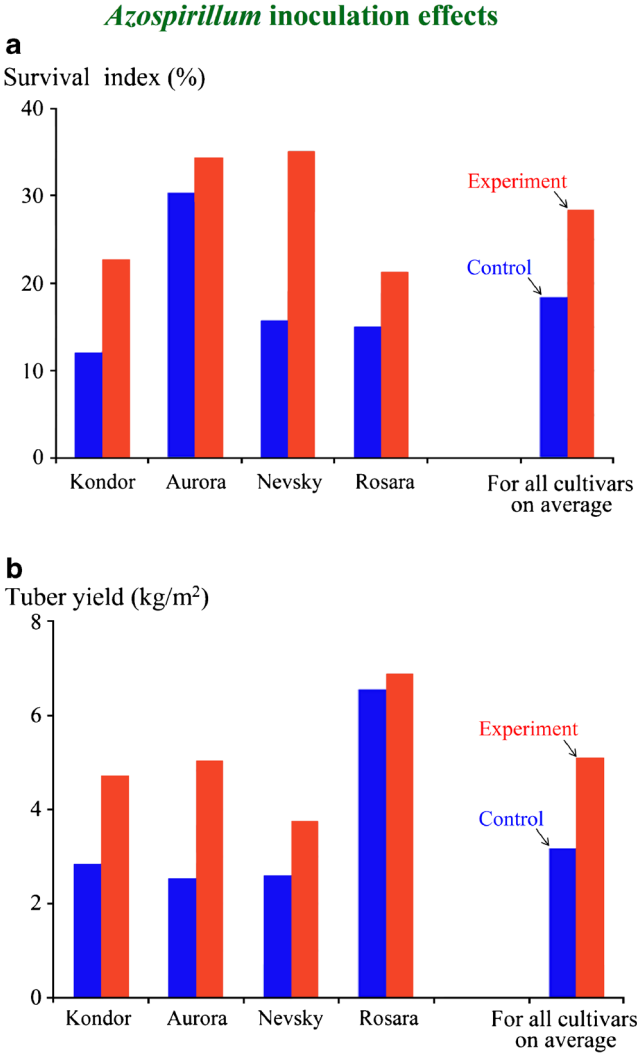

Fig. 3 *Azospirillum* inoculation in vitro significantly increases potato survival in the field (**a**) and tuber yield (**b**). According to the statistical treatment described in Section 2.7, the differences between control and experimental results were significant ($p \leq 0.05$) for all cultivars individually (except for Rosara in Fig. 3b) and on average

de-Bashan 2010; Evseeva et al. 2011). Ultimately, this leads to better rooting of microclones in pot soil, better survival in the field, accelerated biomass accumulation, and increased tuber yield.

In summary, this study is the first to have reported systematic observations of effects of *Azospirillum* inoculation during the microclonal propagation of potato in vitro throughout the production process, from growth of microclones to survival of planting material in soil ex vitro to harvesting. It has been established that *Azospirillum* inoculation of potato microclones not only improves the quality of the in vitro-produced planting material but also significantly increases minituber yield ex vitro through enhancing plant adaptive capacity under stressful field conditions.

4 Conclusion

Microclonal propagation in vitro, used actively in the production of healthy planting material of food and ornamental plants, needs further improvement to increase the growth rates of microclones and enhance regenerant survivability ex vitro. A promising approach to this end is to inoculate in vitro-micropropagated plants with plant growth-promoting rhizobacteria. Several authors have described a positive effect of methylobacteria and pseudomonads on the development of in vitro-micropropagated plants when cultured in vitro and ex vitro.

Of particular interest from this standpoint are associative nitrogen-fixing bacteria of the genus *Azospirillum*, which occur widely in diverse climatic zones and exert positive effects when in ectosymbiotic relationships with many plant species and cultivars. However, we are not aware of any published studies reporting systematic observations of effects of *Azospirillum* inoculation during the microclonal propagation of potato in vitro throughout the production process, from growth of microclones to survival of planting material in soil ex vitro to harvesting. Here, we have demonstrated for the first time that *Azospirillum* inoculation of potato microclones in vitro not only improves the quality of the produced planting material but also significantly increases minituber yield ex vitro through enhancing plant adaptive capacity under stressful field conditions. This might serve as a way to improve the agrobiotechnology of producing potato seed material of significantly better quality.

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