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Research Note

The effect of biostimulants on parsnip seed germination and initial growth

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Abstract

Biostimulants are used for seed quality and performance improvement. However, the impact of biostimulants on parsnip seed quality has not been examined. Slow germination of parsnip is often further impeded by stress conditions. This study therefore aimed to assess whether treatments with different biostimulants could enhance seed germination and the initial seedling growth of parsnip. Amino acid and micronutrient-based biostimulants (0.2% solutions (v/v) of Technokel Amino Mix® and Megafol®) improved germination of parsnip seeds under optimal conditions, while biostimulant treatments with humic acid, fulvic acid and micronutrients (0.02% solution (v/v) of Organiko®) enhanced the initial growth. Our findings validate the potential of biostimulant application as a seed treatment. Determination of the main physiological causes of parsnip germination enhancement is needed in further research.

Experimental and discussion

Plant biostimulants are used in modern agriculture to maximise crop yield and quality, especially if plants are grown under unfavourable environmental conditions (Popko *et al.*, 2018). Biostimulants may be of natural origin and can reduce the use of agrochemicals and thus mitigate their harmful impact on the environment (Calvo *et al.*, 2014; Du Jardin, 2015). The positive impact of biostimulants has been confirmed in lettuce, tomato, potato, okra, melon and other vegetables grown under various conditions (Shahrajabian *et al.*, 2021; Clément *et al.*, 2023). Biostimulants with amino, fulvic and humic acid were demonstrated to enhance germination rate, photosynthesis, nutrient absorption and crop productivity (Yakhin, 2017; Zhang *et al*., 2021; Ampong *et al.*, 2022; Abdelkader *et al.*, 2023) due to a significant increase in protein content and photosynthetic pigments (Souri *et al.*, 2017), carotenoids and amino acids (Sowmya *et al.*, 2023). The involvement of biostimulants in stress defence mechanisms and hormone metabolism was also reported (Sowmya *et al.*, 2023). Biostimulants are usually applied using two methods: direct soil incorporation and foliar spraying (Shahrajabian *et al*., 2021). The current research focused on seed treatment as a reliable, eco-friendly and cost-effective alternative.

To the best of our knowledge, there is no information on the effects of biostimulants on the seed quality of parsnip (*Pastinaca sativa* L.). Parsnip is a vegetable that germinates very slowly, over up to 30 days, and the seeds quickly lose germination potential after the first year of storage. This study aimed to determine the effect of seed treatments with biostimulants composed of amino acids, humic and fulvic acid chelated with micro-nutrients, on seed germination and initial growth. The following plant biostimulants were tested: 0.2 and 2% amino acid and micronutrient solutions (v/v) of Technokel Amino Mix® (AgriTechno, Serbia); 0.2% amino acid, vitamins and betaine solution (v/v) of Megafol® (Valagro S.p.A., Italia); and 0.02% humic acid, fulvic acid and micronutrient solution (v/v) of Organiko® (BioGenesis doo, Serbia). Two lots of parsnip seeds of cv. NS Lala were obtained from the Department of Vegetable and Alternative Crops, Institute of Field and Vegetable Crops, Novi Sad, Serbia. Seed lots were produced under rainfed conditions in 2021 (P-2021) and 2022 (P-2022) at the experimental fields Rimski Šančevi, IFVCNS (N 45°19', E 19°50'), and stored until use in paper bags under a controlled temperature of 4 ± 3°C. Seeds were disinfected with a 4% NaClO solution (v/v) for four minutes, rinsed thoroughly with distilled water for two minutes, then treated with the selected preparations for five minutes and air-dried to approximate initial mass. To initiate seed germination, seeds were completely immersed in each respective biostimulant solution for 15 minutes. The seeds were placed into Petri plates immediately after soaking. Seeds soaked in water were used as control.

Germination test: Working samples consisted of three replicates of 100 seeds. Seeds were placed in Petri dishes (15 cm-diameter) between moistened filter paper at a temperature regime of 20↔30°C, with a photoperiod of 16 hours light per day in a germination chamber. Germination first count was determined on the 6th day by counting only the normal seedlings, while the final germination and the abnormal seedlings were recorded 28 days after seed placement in the Petri dishes (ISTA, 2022).

Seedling growth and biomass accumulation: To obtain parsnip seedling growth, 25 seeds were placed in a rolled moistened filter paper, kept in plastic bags and placed in a germination chamber, under the same conditions as in the germination test. Shoot and root length of 10 seedlings were determined on the 6th and 10th days after seed placement in the filter paper, using a ruler.

The weight of 10 fresh normal seedlings of parsnip was determined on the 10th day after seed placement in filter paper. Seedlings were then oven-dried for 24 hours at 80°C, and dry weight was determined.

Germination-related parameters: Mean germination time (MGT) was determined according to the procedure explained by Ellis and Roberts (1981), using the following formula:

$$MGT = \Sigma(t \times n) / \Sigma n$$

Where t is the time from the beginning of the germination test in days and n is the number of newly germinated seeds at time t.

Mean germination rate (MGR) was calculated using the formula of Ranal *et al.* (2009):

$$MGR = 1 / MGT$$

The seedling vigour index (SVI) was determined using the formula of Abdul-Baki and Anderson (1973):

$$SVI = SL \times FG$$

Where SL is seedling length (cm) and FG is final germination (%).

Growth-related parameters: Shoot elongation rate (SER) and root elongation rate (RER) were obtained according to the procedure of Channaoiu *et al.* (2019) using the following formulae:

$$SER = (SLE - SLS) / (TE - TS)$$

$$RER = (RLE - RLS) / (TE - TS)$$

Where SLE and RLE are the shoot and root length of seedlings (mm) at the start of the measurement period (six days after sowing); SLS and RLS are shoot and root length of seedlings (mm) at the end of the measurement period (10 days after sowing); and TE-TS is the duration between measurement periods (i.e., four days).

Statistical analysis: The experimental design was a completely randomised design (CRD) with four treatments and control. The data were subjected to analysis of variance (ANOVA), followed by means separation according to Duncan´s multiple range test (DMRT) ($p \leq 0.05$) using STATISTICA 10 software (StatSoft Inc., Tulsa, OK, USA). For germination first count, final germination and abnormal seedlings, data were arc-sine square root transformed prior to ANOVA.

The results showed that the biostimulants significantly improved the germination-related parameters in both of the parsnip seed lots in comparison to the control (table 1). However, the response of the two seed lots to priming treatments differed. Although all the treatments improved seed quality, Technokel® had the greatest effect on seed performance of parsnip seed lot 1, while seed lot 2 showed highest percentage germination after treatment with Megafol®. It is surmised that both solutions improved the vigour of parsnip seeds due to their multiple metabolic, structural, and transport functions in plants (Paleckiene *et al*., 2007; Popko *et al*., 2018). Previous studies confirmed the beneficial effect of Megafol® on bell pepper, celery, parsley and leek seed germination (Yildirim *et al*., 2002; Tkalec *et al*., 2010), which is in agreement with the results obtained in this study. Furthermore, the study demonstrated that the treatment with amino acid had a beneficial effect on the quality parameters of onion, cauliflower and broccoli seeds (Abdelkader *et al*., 2023).

Table 1. Results of seed germination and germination-related parameters of parsnip seeds treated with biostimulants.

Treatment	Germination first count (%)	Final germination (%)	Abnormal seedlings (%)	Seedling vigour index	Mean germination time (days)	Mean germination rate
	Seed Lot 1 (cv. NS Lala, P-2021)					
Control	2 c	40 c	0.7 a	191.5 c	14.3 a	0.070 b
0.2% Technokel®	4 b	44 a	0.0 a	249.0 a	12.8 b	0.078 a
2% Technokel®	5 ab	43 ab	0.3 a	232.3 b	12.5 b	0.080 a
0.2% Megafol®	5 ab	42 b	0.3 a	230.0 b	12.7 b	0.079 a
0.02% Organiko®	7 a	43 ab	0.7 a	253.4 a	12.4 b	0.081 a
	Seed Lot 2 (cv. NS Lala, P-2022)					
Control	4 c	76 c	0.0 b	383.3 c	14.0 a	0.071 b
0.2% Technokel®	4 c	83 b	0.0 b	445.9 b	13.1 b	0.083 a
2% Technokel®	7 bc	79 bc	0.0 b	442.7 b	11.7 b	0.086 a
0.2% Megafol®	8 b	87 a	0.7 a	471.9 a	11.6 b	0.087 a
0.02% Organiko®	10 a	78 c	0.0 b	433.3 b	11.7 b	0.085 a
C	***	***	ns	***	**	**
T	***	***	ns	***	***	***
C × T	ns	***	ns	***	ns	ns

*Means (n = 3) with different letters in the column are significantly different ($p \leq 0.05$, Duncan's multiple range test); *$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, ns = not significant.

The biostimulants had a positive effect on the growth-related parameters compared to the control (table 2). Organiko® stood out for its positive impact on shoot growth, fresh biomass accumulation and shoot elongation rate in both of the parsnip seed lots. Similar results were also obtained on pea, yarrow and lettuce in response to fulvic acid and humic acid treatment (Bayat *et al.*, 2021; Lüdtke *et al.*, 2021; Kamran *et al.*, 2023). Previous studies showed that biostimulants increase nutrient transport by influencing the cell membrane, promote photosynthesis by increasing the effectiveness of photosystem II and reduce cell damage by reducing the production of reactive oxygen species and enhancing the activity of antioxidant species (Fang *et al.*, 2020; Kamran *et al.*, 2021). Additionally, 0.2% Technokel® and Organiko® solution was found to be the most effective, in terms of increasing the root elongation rate of parsnip seed lot 1, due to the beneficial effects of amino acids on plant growth via their structure as protein units, essential in glutamine biosynthesis and plant hormone metabolism (Abdelkader *et al.*, 2023).

The selected biostimulants may cause many physiological changes that improve parsnip seed vigour. For this reason, further studies are needed to establish the effect of biostimulants on physiological processes in parsnip seeds and to optimise the seed treatment method.

Table 2. Results of plant growth and growth-related parameters of parsnip seeds treated with biostimulants.

Treatment	Shoot length (mm)	Root length (mm)	Fresh seedling weight (g)	Dry seedling weight (g)	Shoot elongation rate (mm day^{-1})	Root elongation rate (mm day^{-1})
	Seed Lot 1 (cv. NS Lala, P-2021)					
Control	14.30 c	34.00 c	0.159 d	0.030 c	1.11 b	3.05 cd
0.2% Technokel®	17.20 b	39.83 a	0.185 ab	0.038 a	1.45 a	3.62 a
2% Technokel®	18.50 b	35.97 b	0.182 b	0.037 a	1.39 a	2.84 d
0.2% Megafol®	18.27 b	36.47 b	0.168 c	0.035 b	1.52 a	3.34 b
0.02% Organiko®	20.00 a	39.40 a	0.193 a	0.034 b	1.52 a	3.18 bc
	Seed Lot 2 (cv. NS Lala, P-2022)					
Control	17.33 c	33.13 b	0.167 c	0.027 c	1.36 c	2.80 b
0.2% Technokel®	18.1 bc	35.40 a	0.174 bc	0.029 b	1.44 bc	3.06 a
2% Technokel®	19.47 a	36.37 a	0.184 b	0.028 bc	1.59 ab	3.18 a
0.2% Megafol®	18.40 b	35.83 a	0.179 b	0.029 b	1.48 bc	3.11 a
0.02% Organiko®	20.10 a	35.47 a	0.203 a	0.031 a	1.65 a	3.02 a
C	***	***	ns	***	**	**
T	***	***	***	***	***	***
C × T	**	***	**	***	*	***

*Means (n = 3) with different letters in the column are significantly different ($p \leq 0.05$, Duncan's multiple range test); *$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, ns = not significant.

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