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Application of Plant Aqueous Extracts on Yield and Quality Parameters of Soybean Seeds (*Glycine max* L.)

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ABSTRACT

Background: In order to reduce the use of synthetic and chemical agents in agriculture, more and more research is turning to ecological, more environmentally friendly methods. Plant aqueous extracts are products that can be a significant source of various elements, depending on the type and quality of soil on which the plant species from which the solution is prepared is grown.

Methods: The aim of this study was to investigate the influence of aqueous extracts of different plant species on the yield and quality parameters of soybean seeds (*Glycine max* L.). Aqueous extracts of: nettle, nettle+comfrey, banana, banana peel, onion, willow and soybeans were used foliarly. The 1st foliar treatment plants was done when first flowers opened and the 2nd treatment was done when first pod reached final length.

Result: The effect of aqueous extracts depends on the agroecological conditions and the analyzed traits. In 2020 the greatest effect was achieved on the free proline, SOD, Px and CAT. In 2021 the application of certain aqueous extracts had a significant effect on the yield, gerimination energy, germination percentage and vigour seed.

Key words: Aqueous extract, Comfrey, Onion, Soybean, Willow.

INTRODUCTION

During the last decades reliance on the chemicals have led to several problems; increasing incidence of pesticide poisoning of the farmers, reduces soil biodiversity, mammalian toxicity, development of insecticide resistance, causes bio magnification of pesticides, presence of insecticidal residues on marketed vegetables and fruits, hazards to other non-target organisms in the ecosystem and pollinators decline (Dangi and Verma, 2021). In order to reduce the use of synthetic and chemical agents in agriculture, more and more research is turning to ecological, more environmentally friendly methods. There is an increasing number of research aimed at finding the application of alternative measures in plant production in order to avoid unwanted consequences (Miladinov *et al*., 2020a; Mamlic *et al*., 2021). Plant aqueous extracts of certain plants are increasingly used for fertilization in organic production (Kuchlan *et al*., 2017). Plant aqueous extracts can be a significant source of various nutrients. The amount of nutrients depends on the plant species from which the aqueous extract is made and the land on which the plant species grew. Also, plant aqueous extracts are partially insecticides and fungicides due to bioactive chemicals found in the prepared treatment solution (Abdurhman *et al*., 2022). Most of the plant aqueous extracts used are largely the result of traditional knowledge, passed down from generation to generation. Plant material for the management of aqueous extracts is easily available and their application does not economically burden plant production like foliar commercial fertilizers (Chukwuka *et al*., 2014).

The objective of present study was to determine the efficacy of plant aqueous extracts on yield and soybean seed quality parameters.

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MATERIALS AND METHODS

The experiment was conducted from April 2020 to November 2021 at the Institute for Field and Vegetable Crops in Novi Sad, Serbia. Research was conducted with a soybean (*Glycine max* L.) cultivar 'NS Apolo'.

Weather conditions

An analysis of the amount of precipitation during the vegetative period in 2020 and 2021 showed that about 45.12% more rain fell in 2020. Not only the amount of precipitation, but also its distribution differed between the two experimental years. Apart from precipitation during the vegetation season in 2020 and 2021, there were also differences in the air temperature (www.hidmet.gov.rs, 2022)

(Fig 1). During the vegetation period in 2020 there was 231.7 mm more precipitation, or 45.12% more than in 2021. An analysis of the water needs of plants and the amount of precipitation by individual years revealed that in 2020, the needs were 462.1 mm and the available water amount was 471.2 mm, while the water needs in 2021 were higher due to high temperatures and amounted to 480.1 mm, while only 236.2 mm of water was available to the plants. In 2020, there was no water deficit and in 2021-243.8 mm (Table 1).

Plant materials

The experiment was set up as a randomized block design (RCBD) with four replications under the conditions of dry farming. Plot size was 10 m^2. Inter-row spacing of 50 cm and intra-row spacing of 4.5 cm was applied. Each year the plots with soybean plants were rotated with maize (*Zea mays* L.). The 1st foliar treatment plants was done at growth stage 6: flowering (main shoot), when first flowers opened (sporadically in population) (BBCH 60 600) and the 2nd treatment was done at growth stage 7: development of fruits and seeds when first pod reached final length (15-20 mm) (BBCH 79 709) (Munger *et al*., 1997). Treatments with the foliar application of aqueous extracts were as follows: control-without any foliar spray; an aqueous extract of nettle (*Urtica dioica* L.), an aqueous extract of nettle (*Urtica dioica* L.) and comfrey (*Symphytum officinale* L.) prepared a ratio of 3:1; an aqueous extract of the banana fruit (*Musa* × *paradisiaca*); an aqueous extract of banana peel (*Musa* × *paradisiaca*); an aqueous extract of onion bulb leaves (*Allium cepa* L.); an aqueous willow twings extract (*Sali* × *matsudana*); an aqueous extract of top parts of soybean plants (*Glycine max* L.).

For the preparation of plant extracts, one kilogram of plant material was used, which was cut into small pieces, the size of 1-4 cm. After cutting, the plant material was submerged in 10 l of rainwater and left to ferment for all extracts 30 days with daily stirring. The obtained plant extract was filtered through gauze and left to stand at a temperature of 18-22°C in glass bottles, hermetically sealed. Prior to the foliar treatment of soybean plants, the aqueous extract was diluted with distilled water in a ratio of 1:15. No fertilizers or pesticides were applied during the experiment. Weeds were destroyed mechanically. There were no significant diseases and pests.

After basic plot harvesting, grain weight and moisture were measured and the yield was calculated in kg ha^{-1} and adjusted to a grain moisture content of 14%. Seed germination was performed under laboratory conditions immediately after harvest using the standard laboratory method. The standard laboratory test was performed for 4 × 100 seeds using sand. The incubation period was 8 days at 25°C and 95% relative humidity (ISTA, 2019). The germination energy (GE) was recorded after 4 days and the germination percentage (GP) after 8 days. Vigour index (VI) was calculated according to the formula VI= GP × SL, where GP= Germination percentage and SL= Seedling length after 8 days (Abdul-Baki *et al*., 1973). After 8 days, 4 × 10 average seedlings were taken from each treatment and used for analysis.

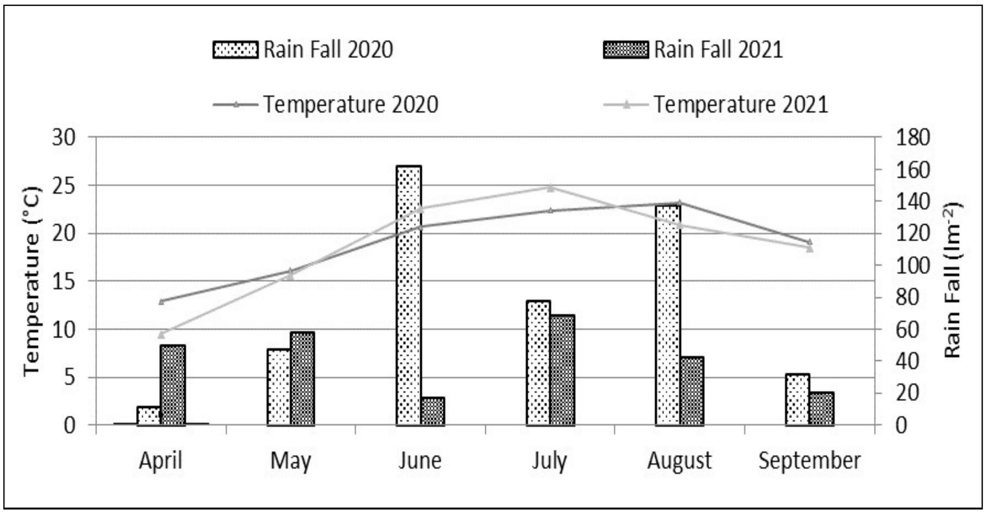

Fig 1: Monthly temperatures and precipitation the vegetation season in 2020 and 2021.

Table 1: Potential and actual evapotranspiration (mm) during soybean vegetation in 2020 and 2021.

Year	SM	PV	PE	ET	PD	DS
2020	42.31	415.4	462.1	471.2	+9.1	-
2021	46.01	190.2	480.1	236.2	-243.8	19.06.

SM- soil moisture content at sowing, PV- Precipitation during vegetation period, PE- Potential evapotranspiration, ET- Evapotranspiration, PD- Precipitation deficit; DS- Drought start (date).

Malondialdehyde (MDA) was estimated following Placer *et al*. (1966) protocol. MDA from soybean fresh seedlings was done by using solution of TBA, TCA and PCA reagents. Soybean fresh seedlings (0.5 g) were homogenized in a mortar with 4.5 ml extraction solution and incubated in a water bath at 90°C for 20 min. After incubation, the solution was cooled to stop the reaction and centrifuged for 10 min at 5500 r min^{-1}. The concentration was determined spectrophotometrically at 532 nm.

SOD activity (U mg $protein^{-1}$) was determined using spectrophotometer method, on the basis of reaction of autooxidation of adrenaline to adrenochrome. Change of adrenaline solution absorbance was measured at 480 nm in carbonate buffer pH 10.2. The activity of Px (U mg $protein^{-1}$) was determined on the basis of transformation of guaiacol into tetraguaiacol and by measuring change of absorbance at 436 nm in phosphate buffer (Matkovics *et al*., 1989).

Catalase (CAT) determined by addition of 50 µL of crude enzyme extract to 2.95 ml of reaction medium consisting of 100 mM potassium phosphate buffer, pH 6.8 and 12.5 mM H_2O_2. The decrease in absorbance by H_2O_2 consumption was measured for the two minutes of reaction at 240 nm at a temperature of 25°C. Enzyme activity was calculated using the molar extinction coefficient of 36 M^{-1} m^{-1} and expressed in µmol of H_2O_2 min^{-1} mg^{-1} protein (Anderson *et al*., 1995). Free proline concentration was determined by the method of Bates *et al*. (1973). Soybean fresh seedlings (1 g) were extracted with 3% sulphosalicylic acid. The extracts (2 ml) were kept for 1 h in boiling water by adding 2 ml ninhydrin and 2 ml glacial acetic acid, after which cold toluene (4 ml) was added. Free proline content was measured by a spectrophotometer at 520 nm and calculated as µmol × g^{-1} FW against standard proline.

Statistical analysis

The data were analysed using one-way analysis of variance (ANOVA). The mean values from treatments were compared using Tukey's HSD (honestly significant difference). The AMMI model was used to analyse the interaction. AMMI analysis of variance and AMMI1 biplot were done using software GenStat (VSN International, UK).

RESULTS AND DISCUSSION

Soybean seed yield

The foliar-applied plant aqueous extracts had a significant impact on soybean yield in both years of the study (Table 2). In 2020, plant aqueous extracts increased the yield from 10.13%-15.67% and in 2021 from 13.26-20.79%. In 2020 there was no precipitation deficit, i.e. even an excess of 9.1 mm was recorded, while in 2021 the deficit was 256 mm with a dry period of 93 days. Also, in 2020, during the growing season, 17 days were recorded with temperatures above the optimum for normal plant growth and development. In 2021, this period lasted significantly longe. The positive effect of certain effects can be attributed to the chemical composition of the plants from which they are made (Rivera *et al*., 2012).

Seed germination

In 2020, only the application of aqueous extract of willow achieved a greatest effect. GE increased by 4.21%, GP by 4.08% and VI by 13.24%. However, the negative effect was significantly higher especially for GE. Using nettle+comfrey aqueous extract, GE was reduced by 15.38%, GP by 7.45% and VI by 27.50% compared to the control. Mahdi (2010) suggest that the improvement of seed germination, after the application of extract of willow is due to less contamination of soybean seeds by pathogenic organisms due to the effect of salicylic acid and due to the decreased MDA. Aqueous extract of willow is rich in auxins that have a positive effect on different physiological processes in the plant, from seed germination to flowering (Liu *et al*., 2017). In 2021, the application of the aqueous extract achieved the greatest effect, both positive and negative. The greatest effect was achieved with the use of extract willow this year as well. GE increased by 16.67%, GP by 9.28% and VI by 24.54%. The results showed that the application of aqueous

Table 2: Influence of spray with plant aqueous extract on seed yield and seed germination.

Treatment	Yield (kg ha^{-1})		Germination energy (%)		Germination percentage (%)		Vigour index	
	2020	2021	2020	2021	2020	2021	2020	2021
Control	3024	1962	91	78	94	88	591.41	431.52
Water	3246^{ns}	2231^{ns}	91^{ns}	82*	94^{ns}	94*	594.37^{ns}	510.11*
Nettle	3478*	2370*	92^{ns}	83*	92^{ns}	92^{ns}	597.77^{ns}	514.65*
Nettle+comfrey	3525**	2412*	77**	42**	87**	67**	428.12**	212.47**
Banana	3586**	2477**	90^{ns}	90**	95^{ns}	94*	675.12**	559.12**
Banana peel	3482**	2335*	91^{ns}	87**	95^{ns}	91ns	641.12^{ns}	521.51*
Onion	3448*	2313*	94*	90**	96*	96**	672.18*	568.24**
Willow	3365*	2262ns	95*	91**	98*	97**	681.39*	571.82**
Soybean	3475*	2297*	85*	70**	91ns	82*	508.11*	304.19**

Note. Different letters in each column represent significant difference at p≤0.05 according to Tukey's HSD.

aqueous extracts has a greater effect on GE, GP and VI in unfavorable agroecological conditions. Miladinov *et al*. (2020b) also point out that the application of foliar treatments on GP has a greater impact in years with a lack of precipitation than in years with sufficient amounts of precipitation during the soybean growing season. The same authors point out that foliar application, in addition to having a positive effect on GP, can also have a negative effect, depending on the agroecological conditions and the solution with which the plant is treated. The negative effect of the aqueous extract of nettle+comfrey is significantly greater than in 2020. GE decreased by 46.15%, GP by 23.86% and VI by 50.76% compared to the control. There are numerous studies in which it was found that the use of certain aqueous extracts can significantly increase but also reduce GP. Petrova *et al*. (2015) found seeds of *Triticum aestivum* L., *Sorghum halepense* L., *Chenopodium album* L., *Cynodon dactylon* L. and *Rumex crispus* L. negative impact of application of aqueous extracts on GP and seedling growth. The aqueous extracts showed significant negative effects, primarily the application of the highest concentration of 5%, which in some cases reduced the GP and growth of seedlings up to 100%.

Lipid peroxidation intensity and free proline content in soybean seedlings

In 2020, plant aqueous extracts only influenced an increase in the MDA. With the use of aqueous extract of nettle+comfrey and soybean, the MDA increased by 33.34% and 25.55%, respectively (Table 3). Cai *et al*. (2011) point out that the decrease in seed germination is due to an increase in the intensity of lipid peroxidation, *i.e*. an increased accumulation of MDA, whose content is an indicator of cell membrane damage and is associated with uncontrolled reactive oxygen species (ROS) accumulation. In 2021, the application of plant aqueous extracts had a greater impact on the MDA. With the use of aqueous extracts of willow, onion and banana, the MDA was reduced from 19.23%-30.72%. The reduction of MDA due to the application of plant aqueous extracts is a consequence of the increased activity of antioxidants and the result of all this is improved seed quality (Cai *et al*., 2011). Application of aqueous extracts of nettle+comfrey and soybean increased the MDA by 32.26% and 17.59%, respectively. Significantly higher accumulation of MDA in soybean seedlings in plants treated with nettle+comfrey indicate the fact that the stress caused by allelopathic substances was strong enough and the pure effects of antioxidant enzymes could not prevent the oxidative burst and induction of lipid peroxidation (Mutlu-Durak and Yildiz Kutman, 2021). In 2020, with the application of aqueous extracts of onion and willow, the content of free proline increased by 27.71% and 25.45%, respectively, while the application of aqueous extracts of nettle+comfrey and soybean reduced the content of free proline by 47.39% and 35.54%, respectively. In 2021, plant aqueous extracts had an effect only on the increase of free proline content. By using aqueous extracts of banana fruit, onion and willow, the content of free proline increased from 10.23%-20.20%. Reduction observed in the levels of free proline may be due to the mobilization of free proline in order to combat the threat posed by the aqueous extract (Geethika Sai *et al*., 2022). The role of proline in plants under stress includes protection of the structure of enzymes and proteins, maintenance membrane integrity, protects against reactive oxygen species, also serves as a pool of carbon and nitrogen source for the plant after the plant has undergone stress (Hameed *et al*., 2012).

Antioxidant activity in soybean seedlings

In 2020, only the application of aqueous extracts of banana, willow and onion had a significant impact on increasing antioxidant activity. By using these plant extracts, SOD activity increased from 32.38%-35.38%, Px from 28.85%-38.42% and CAT from 18.24%-24.84% (Table 4). The negative effect was less. Using the aqueous extract of nettle+comfrey and soybean reduced SOD by 35.71% and 23.81% respectively, Px by 20.09% and 19.69% respectively and CAT by 14.97% and 15.82% respectively. In 2021, only the application of aqueous extracts from banana, willow and onion had a positive effect on increasing antioxidant activity.

Table 3: Influence of spray with plant aqueous extract on MDA and free proline content in soybean seedlings.

Treatment	MDA (mmol × g^{-1} FW)		Free proline (µmol × g^{-1} FW)	
	2020	2021	2020	2021
Control	27.32	42.12	2.87	3.95
Water	25.80^{ns}	36.18^{ns}	2.90^{ns}	3.72^{ns}
Nettle	26.20^{ns}	38.05^{ns}	2.69^{ns}	3.50^{ns}
Nettle+comfrey	42.12**	62.18**	1.51**	3.55^{ns}
Banana	26.90^{ns}	29.18**	3.10^{ns}	4.40*
Banana peel	25.17^{ns}	37.11^{ns}	2.71^{ns}	4.02^{ns}
Onion	23.20^{ns}	32.15*	3.85*	4.80*
Willow	24.18^{ns}	34.02*	3.97*	4.95*
Soybean	37.34*	51.11*	1.85*	3.60^{ns}

Note. Different letters in each column represent significant difference at $p \leq 0.05$ according to Tukey's HSD.

Table 4: Influence of spray with plant aqueous extract on antioxidant activity in soybean seedlings.

Treatment	SOD (U mg protein^{-1})		P_x (U mg protein^{-1})		CAT (U mg protein^{-1})	
	2020	2021	2020	2021	2020	2021
Control	4.2	3.9	25.4	29.4	35.4	37.1
Water	4.5^{ns}	4.1^{ns}	26.2^{ns}	32.3^{ns}	34.2^{ns}	37.5^{ns}
Nettle	4.5^{ns}	3.8^{ns}	27.1^{ns}	29.1^{ns}	37.1^{ns}	36.1^{ns}
Nettle+comfrey	2.7*	3.2*	20.3*	22.4**	30.1*	31.4**
Banana	6.2*	5.1*	35.7*	41.8**	43.3**	45.1**
Banana peel	4.4^{ns}	3.9^{ns}	28.6^{ns}	29.6^{ns}	33.8^{ns}	35.1^{ns}
Onion	6.5*	5.2*	39.7**	47.7**	46.4**	46.9**
Willow	6.4*	5.3*	41.2**	46.2**	47.1**	46.8**
Soybean	3.2*	2.9*	20.4*	24.1*	29.8*	31.7*

Note. Different letters in each column represent significant difference at $p \leq 0.05$ according to Tukey's HSD.

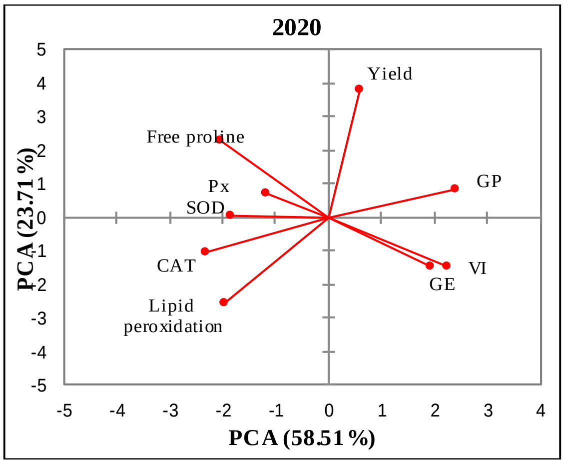

Fig 2: Treatment by trait (TT) biplot: plant aqueous extract on seed yield and quality parameters of soybean seed in 2020 seed germination.

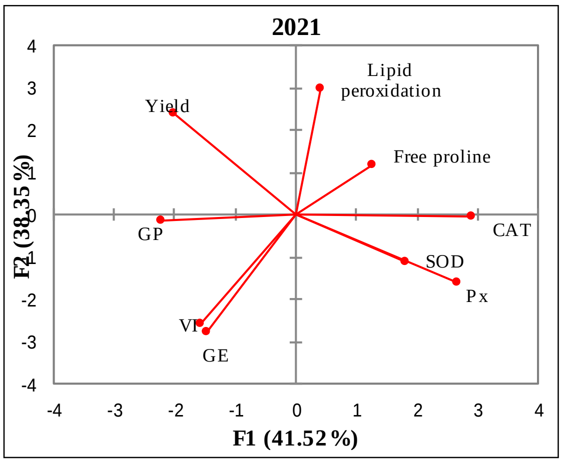

Fig 3: Treatment by trait (TT) biplot: plant aqueous extract on seed yield and quality parameters of soybean seed in 2021 seed germination.

SOD activity increased from 23.53%-26.42%, Px from 29.67%-38.36% and CAT from 17.74%-20.90%. Application of aqueous extracts of nettle+comfrey and soybean affected the reduction of antioxidant activity, similar to the year 2020. SOD was reduced by 17.95% or 25.64%, Px by 23.81% or 18.03% and CAT by 15.36% or 14.56%. Plants have developed a complex system of enzymatic and non-enzymatic antioxidant protection to neutralize the effects of ROS in cells: superoxide dismutase (SOD), catalase (CAT) and peroxidase (Px) (Sharma *et al*., 2012). SOD, considered the first line of defense at the cell level, acts in protection against the superoxide radical (O_2), disproportionate it to hydrogen peroxide (H_2O_2) and oxygen (Saisanthosh *et al*., 2018). H_2O_2 is detoxified into molecular oxygen and water by two different systems: the glutathione peroxidase system, which is the first line of defense against H_2O_2, or CAT and PX, which also act in reduction of hydrogen peroxide (Matos *et al*., 2014). The results of the analysis showed that plants that were treated with plant aqueous extracts that led to increases in GE, GP and VI, had a significantly higher content of SOD, Px and CAT. Lizcano *et al*. (2012) point out that the application of certain plant aqueous extracts can have important roles due to their antioxidant behavior and their oral consumption as aqueous infusions is supported for possible prevention of diseases associated with oxidative stress. The results showed that the use of aqueous extracts of onion and willow increases the content of SOD, Px and CAT which inactivate active forms of oxygen, preventing superoxidation of lipids and damage to the cells (Mutlu-Durak and Yildiz Kutman, 2021).

In the TT biplot, a vector is drawn from the biplot origin to each marker of the tester (treatment) to facilitate visualization of the relationships between and among the measured traits as well as treatments. In 2020. year (Fig 2, 3), 58.51% of total variation was explained with first (PC1) and 23.71% with second principal component (PC2). The biplot indicates a positive relationship between the vectors of two germination traits if the angle value (>0 and < 90°)

gets narrower and a negative relationship as the angle value (>90 and <180°) gets wider (Yan and Rajcan, 2002). Biplot revealed strong positive association among VI and GE, as indicated by the acute angles between their vectors. These traits were negatively associated with free proline and Px, as indicated by the obtuse angle. Also, a significant correlation was established between yield and GP. However, these two traits were negatively related to MDA, *i.e* CAT. Also, a significant correlation was established between yield and GP. However, these two traits were negatively related to MDA, *i.e* CAT. In 2021 year (Fig 3), 41.52% of total variation was explained with first (PC1) and 38.35% with second principal component (PC2). Biplot revealed strong positive association among VI and GE. Among, these traits were negatively associated with free proline and MDA. Yield was strongly negatively correlated with Px and SOD and GP with CAT.

CONCLUSION

The effect of aqueous extracts depends on the agroecological conditions and the analyzed traits. In 2020 the greatest effect was achieved on the free proline, SOD, Px and CAT. In 2021 the application of certain aqueous extracts had a significant effect on the yield, gerimination energy, germination percentage and vigour seed. Therefore, further research is needed in order to find a plant aqueous extracts that would have a favorable effect on all plant properties, both in favorable and unfavorable agroecological conditions. Taking into account the absence and/or insufficient number of studies concerning the effect of investigated plant species aqueous extracts on yield, germination parametars and biohemic traits of soybean seedlings, the synergistic or antagonistic effects of some phytochemicals are not excluded. Such a topic may be elucidated by future research. Therefore, we can conclude that there is no universal use of one single primer, as it may not be suitable for each particular variety and can ultimately lead to a decrease in the quality of seed.

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Conflict of interest

1. All co-authors agree to publication of the submitted manuscript.
2. All co-authors agree to the pposting of the full text of this work on the journal web page and to the inclusion of references in the databases accessible on the internet.
3. No reserach of the other reserachers were used in the submitted manuscript without their consent, proper citation or acknowledgement of their cooperation or materila provided.
4. The results (or any part of them) used in the manuscript have neither been sent for publication to any othor journal nor have already been published.
5. The authors report no conflict of interest.
6. The authors alone are responsible for the content and writing of the paper.
7. After publication of the paper Legume Research will be the copyright owner.

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