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MORPHOLOGICAL AND BIOCHEMICAL PARAMETERS IN CHEMICALLY ELICITED RYE SPROUTS

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ABSTRACT: The growth and chemical composition of edible plantlets, also named microgreens, can be qualitatively and quantitatively influenced by exogenous factors, allowing the opportunity to obtain improved products. The effects of spent brewery yeast on rye microgreen growth and composition and the potential use as an elicitor is not reported. Also, the effects of ascorbic acid elicitation are largely unexplored in rye plantlets, while it is known that ascorbic acid may induce higher levels of phenolic substances biosynthesis in other species. In the present study, rye microgreens elicited with spent yeast presented improved germination, especially after 4 days of cultivation. Increased plantlet length and fresh mass were recorded for 50 % yeast treatment after 8 days of cultivation. Total phenolic contents were enhanced by 10 and 50 % yeast treatment after 4 days of cultivation. Antioxidant activity of rye microgreens were higher than control plantlets at all yeast concentrations after 6 days of cultivation. Ascorbic acid increased the germination percentage at 1000 mg/l and lead to increased plantlet length and biomass at 100 and 500 mg/l after 8 days of cultivation. Total phenolic contents were stimulated after 8 days of cultivation by 1000 mg/l ascorbic acid.

Spent brewery yeast and ascorbic acid may be further investigated as eliciting agents for cultivating rye microgreens with improved growth and bioactive substances contents.

Keywords: rye sprouts, phenolic contents, antioxidant activity

INTRODUCTION:

Consumption of plant germs is a practice long used in food, especially in Asian and Least Developed Countries. However, in the last decades, vegetable germs have penetrated into the preferences and food market of consumers in the western countries, their consumption being constantly increasing. The reason is the chemical composition rich in substances that have a beneficial role in maintaining health, and also, the special taste and texture these germs can offer to their food (Mir et al., 2017). Vegetable germs are unprocessed foods that retain their nutritional and therapeutic qualities, produced most commonly in organic setups. This makes vegetative germs to be considered functional foods, which, in addition to nutrient intake, can positively influence the health of consumers (Janovska et al., 2010).

Elicitors are substances that induce physiological changes in the plant (Swieca et al., 2015). Plants respond to these stressors by activating a series of mechanisms, similar to responses to pathogenic or environmental stimuli, affecting plant metabolism and increasing synthesis of phytochemicals (Zhao et al., 2015a). Elicitors include synthetic as well as natural substances such as plant hormones (jasmonate), polysaccharides (chitosan, alginates, cellulose), secondary metabolites (organic and phenolic acids) etc. (Perez-Balibrea et al., 2011). Such an elicitor may be derived from the normal metabolic processes of the organism but may also be synthesized as a result of fungal, bacterial, viral or herbivorous infections (exogenous elicitors) and in some cases are released from plants attacked by the action of the enzymes of the pathogen (Carvacho et al., 2014).

The first step in the plant's response against elicitor is the perception by receptors located in the plasma membranes of plant cell such as protein kinases. The signal can then stimulate synthesis of different classes of secondary metabolites and the response is mainly dependent on the genetics of the species and its physiological state (Ramirez-Estrada et al., 2016). The elicitor concentration may also induce various plant species responses, making it necessary to find empirically an effective dose and timing. Generally, plants achieve better results in the exponential growth phase, when the concentration of bioactive compounds is higher. Use of elicitors during pre-harvest can be used by the fresh product industry to produce healthier products by improving their nutritional content. Moreover, interest in functional foods has grown over the last decade, with consumers being increasingly concerned about diet and nutrition (Baenas et al., 2014).

Spent brewer yeast is a byproduct that deserves considerable attention due to its large quantity (it is the second largest by-product in the beer industry) and has a rich chemical composition. The predominant chemical element in yeast cells is carbon, which represents less than 50% of the dry mass. Other major elemental components are oxygen (30-35%), nitrogen (5%), hydrogen (5%) and phosphorus (1%). Main macromolecules are proteins (47 %) and carbohydrates (43 %) (Briggs et al., 2004), along with B-vitamins, nucleic acids and minerals (Ferreira et al., 2010). In yeast cell membranes, β-glucans and mannoproteins are found, molecules that are known to bind with cellular receptors and generate immunologic like responses (Bzducha-Wróbel et al., 2013). Spent brewer yeast is generated in a ratio of 1.7-2.3 kg/m^3 of beer and its main uses are food supplementation in livestock (fish and ruminants), substrate for microbial growth or food flavouring (Farcas et al., 2017). Although yeast extracts were used as elicitors for other sprout species,

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the use of spent brewer yeast as an elicitor for rye sprout production was not reported previously.
Ascorbic acid has been chosen also as an elicitor for this study because it is a cellular metabolite that is known to be involved in various signalling and defence mechanisms during biotic and abiotic stress. Therefore, it is assumed that ascorbic acid elicitation can mimic a response to stress in the host, which leads to an increase in the synthesis of phenols that are very beneficial to health (Randhir & Shetty, 2007). Moreover, the use of ascorbic acid as an elicitor for rye sprouts has not been reported previously.
Therefore, the present paper test the hypothesis that spent brewery yeast and ascorbic acid may lead to better growth and total phenolic contents in rye microgreens.

MATERIAL AND METHODS:

For microgreen production, ecological certified rye (*Secale cereale* L.) seeds were used, commercially obtained (Germline, France). Seeds were placed in sterilised Petri dishes, 9 cm diameter, on sterilised filter paper, moistened initially with 3 ml of distilled water or, respectively, solutions of ascorbic acid or of used yeast. Ascorbic acid (Sigma) concentrations used were 100, 500 and 1000 mg/l, according to available literature. Spent brewery yeast slurry was obtained from Bermas brewery, Suceava, Romania. Spent yeast solutions were of 10, 50 and 100% concentrations, diluted with distilled water where required.

Plates were kept under ambient light and temperature regimes and filter papers were moistened when required with the same volume in each plate, usually between 1 and 2 ml/plate. Each treatment comprised 5 plates, holding 50 rye seeds each.

Germination percentage was determined for each treatment, on the 4th and 6th day for yeast and ascorbic acid treatments and also on the 8th day after experiment assembly, for ascorbic acid treatment. Germination percentage was calculated as the number of germinated seeds*100/total number of seeds per plate. Seeds were considered germinated when the radicle protruded at least 1 mm through the seed coat.

For total phenolic and antioxidant activity assessments, microgreens extracts were prepared in 80% (v/v) ethanol solutions, at a ratio of 5 g plant / 95 ml solvent in glass beakers, the extraction being carried out using a horizontal shaker at a speed of 200 rot/min.

Total phenolic content assessment was performed as described in Herald et al. (2012). After centrifugation, 0.1 ml of extracts were mixed with 1 ml of distilled water and 0.1 ml of Folin reagent. After 5 minutes, 0.8 ml of 7.5% Na_2CO_3 were added and allowed to incubate for 60 minutes. Absorbance was read at 760 nm using a Shimadzu UV-mini 1240 spectrophotometer and results were calculated according to a calibration curve prepared using gallic acid and expressed as mg gallic acid/g fresh weight.

Free radical scavenging capacity was assessed using the DPPH method, described in Herald et al. (2012). A 0.1 ml extract volume was mixed with 2.9 ml 60 µM DPPH (Sigma) solution and allowed to incubate for 90 minutes. Absorbance was read at 515 nm and results were expressed as procentual free radical scavenging capacity based on colour inhibition compared to a reagent blank prepared using only DPPH solution and 0.1 ml of 80% ethanol.

RESULTS AND DISCUSSION:

The germination process was positively influenced by both ascorbic acid and spent yeast treatments. The spent yeast led to increases with up to 21.3% compared to untreated seeds, of the number of germinated seeds after 4 days of cultivation at a 10% spent yeast concentration (Fig. 1). After 6 days of cultivation, the highest germination percentages were recorded for 10 % and 50 % spent yeast treated seeds. The ascorbic acid treatments induced higher germination percentages for all tested concentrations (Fig. 1). Compared to untreated seeds, the largest difference was recorded for 1000 mg/l ascorbic acid concentration.

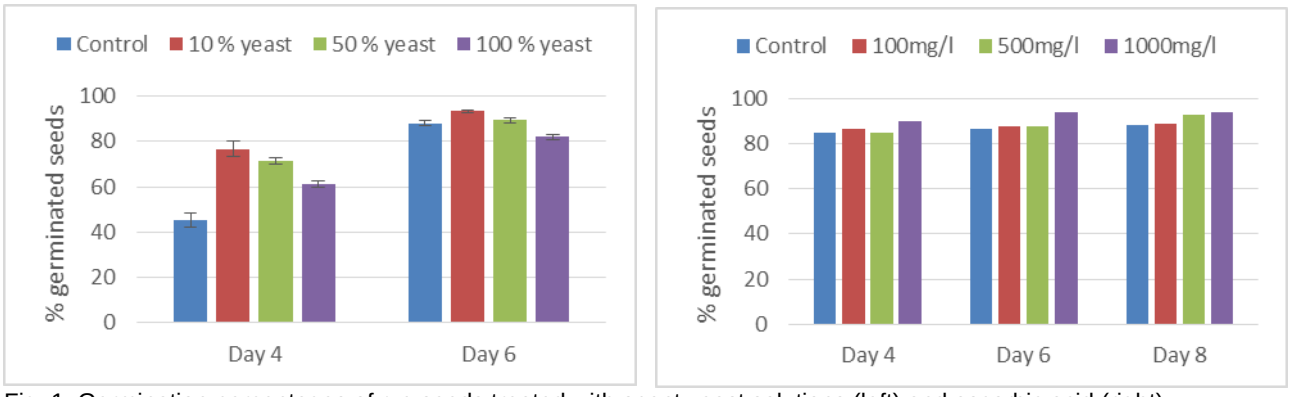

Fig. 1. Germination percentages of rye seeds treated with spent yeast solutions (left) and ascorbic acid (right)

Growth parameters, plantlet length and mass, were also influenced by the treatments. Hypocotyl length was higher after 4 days of cultivation in 100 % spent yeast treated microgreens and after 8 days of cultivation in 50 % yeast treated microgreens (Fig. 2). Ascorbic acid induced higher values of microgreen hypocotyl length after 6 days of cultivation at 100 and 1000 mg/l and after 8 days of cultivation at all concentrations (Fig. 2). Plantlet development, expressed as fresh mass, was stimulated only in the case of 50 % spent yeast and 100 and 500 mg/l ascorbic acid treatments (Table 1).

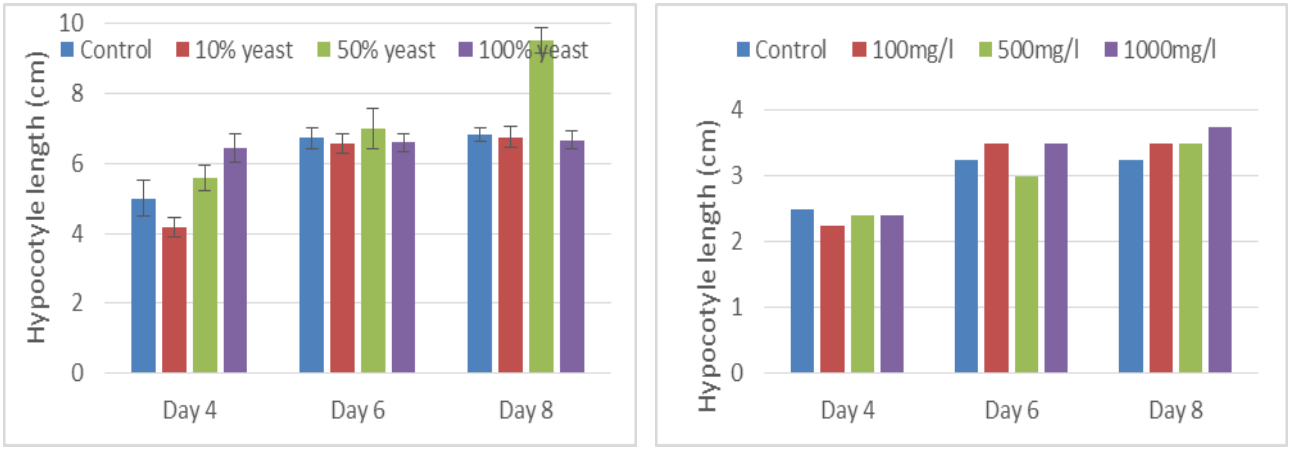

Fig. 2. Hypocotyl length of rye microgreens treated with spent yeast solutions (left) and ascorbic acid (right)

The total phenolic contents were positively influenced compared to untreated microgreens by spent yeast treatments on the 4th day of cultivation at 10 % and 50 % concentrations (Figure 3). On the 6th day, higher phenolic contents were registered for the 100 % yeast treatment. Free radical scavenging activity was higher for treated microgreens only after 6 days of cultivation for all concentrations (Figure 4).

Ascorbic acid did not exert significant influence over the phenolic contents of rye microgreens, except for the 8th day of cultivation, where 1000 mg/l treated plantlets had higher values (Figure 3). Free radical scavenging capacities were higher after 4 and 8 days of cultivation, for 1000 and, respectively, 100 mg/l ascorbic acid solutions (Figure 4).

Tab. 1.
Fresh masses of yeast and ascorbic acid treated microgreens

Treatment (spent yeast)	Fresh mass (g/10 microgreens)	Treatment (ascorbic acid)	Fresh mass (g/10 microgreens)
Control	0.48	**Control**	0.50
10 %	0.46	**100 mg/l**	0.59
50 %	0.51	**500 mg/l**	0.57
100 %	0.35	**1000 mg/l**	0.46

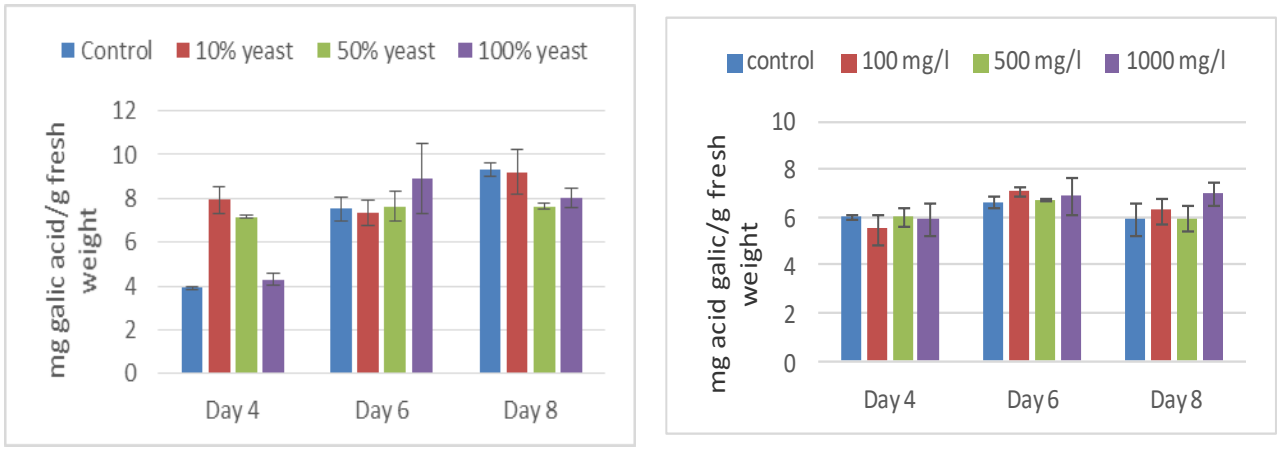

Figure 3. Total phenolic contents of rye microgreens treated with spent yeast solutions (left) and ascorbic acid (right)

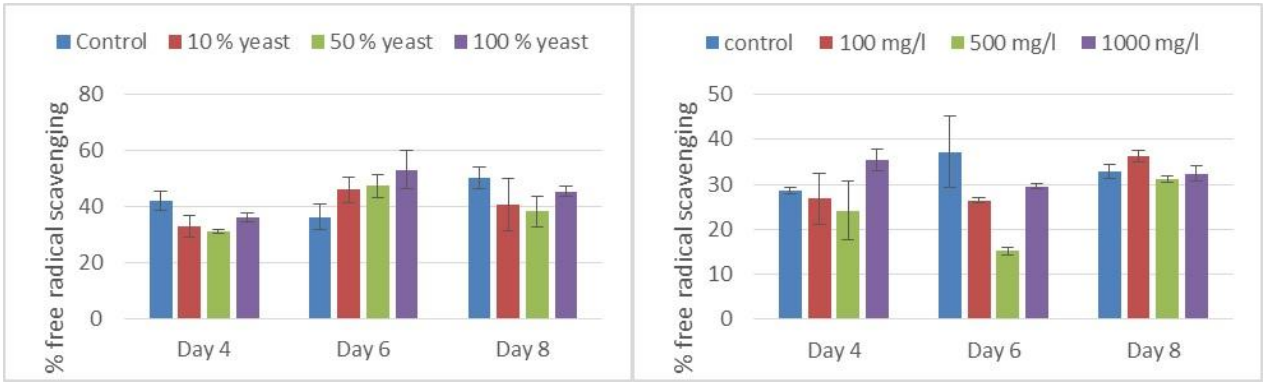

Fig. 4. Free radical scavenging capacity of extracts of spent yeast (left) and ascorbic acid (right) treated rye microgreens

It can be seen that the response of rye plantlets to the elicitors is not dose dependent. Moreover, the effects of treatments are not consistent over the 8 days of experiment. This may be a result of a high variability in seed characteristics, as it is known that the plant response is highly dependent on the genetic traits of plant material (Ramirez-Estrada et al., 2016). In the same time, the response of the plantlets depends also on the chemical composition of the elicitor and on growth conditions. For example, in broccoli sprouts, the total content of phenolics has not been influenced by amino acid fertilization, but has been significantly altered by the application of salicylic acid or methyl jasmonate as the elicitor (Perez-Balibrea et al., 2011). The daily application of methyl jasmonate in brocooli sprouts resulted in increases of 31% in flavonoids, 23% in phenols, and 22% in glucosinolates. The use of 100mM salt solution resulted in a 20% and 40% increase on the third and fifth day of germination of the phenolic content of radish sprouts (Baenas et al., 2014). In bean sprouts, the highest increase in the phenolic compound content was observed after glutamic acid administration for eight days, suggesting that glutamic acid could stimulate the phenylpropanoid pathway that triggers the accumulation of phenolic compounds (Limon et al., 2014).

Natural molecules such as proteins and their hydrolysates may be used as elicitors, as was demonstrated in bean sprouts treated with fish protein hydrolysate and lactoferrin. A seed stimulation of 2 ml / l showed the highest phenolic content on the second day (3.4 mg / g FW), a trend which was maintained for days 3, 4 and 5, higher than that of control sprouts. Lactoferrin was shown to be a better elicitor at a low concentration of 50 ppm on day 3 after the experiment was assembled (Randhir et al., 2002).

In yeast, the most abundant classes of macromolecules are proteins and carbohydrates. The composition of each class of macromolecules in the yeast cell varies according to the physiological state and phase in the growth cycle (Briggs et al., 2004). Protein content has several linked amino acids, including arginine, glycine, histidine, leucine, lysine, methionine, phenylalanine, tryptophan, threonine, tyrosine, and valine, among which precursors of phenylpropanoid, phenolic compound producing pathway, are found. Leucine, lysine and tyrosine are the most abundant aminoacids in yeast cells (Mussatto, 2009).

Saccharomyces cerevisiae extracts may be used as elicitors in sprouts, although their efficiency may vary. Yeast treatment in wheat sprouts had no significant effect (Dziki et al., 2015), while in buckwheat sprouts it lead to 8 % biomass and 1.4 fold flavonoid contents increases (Zhao et al., 2015b), where the effect on phenolic compounds was attributed to the attachment of yeast polysaccharides to receptors on sprout surface and the generation of signals, leading to stimulated phenylalanine ammonia lyase (PAL) activity. Yeast extracts were also an efficient elicitor of phenolic production in broccoli sprouts (Gawlik-Dziki et al., 2013), where it increased the contents of ferulic acid, p-coumaric acid, syryngic acid and kaempferol up to 17 fold. The stimulation of phenolic production by yeast elicitation is considered to take place by reorienting a part of the metabolic carbon (as sucrose), from primary metabolic pathways towards secondary metabolite production, aminoacids and organic acids. This was demonstrated in cell cultures of *Medicago truncatula*, where 5 mg/ml yeast cell wall solutions induced a 2.29 fold accumulation of alanine, 1.40 fold accumulation of aspartic acid, 1.39 fold accumulation of citric acid and 1.56 fold accumulation of sikhimic acid, precursor of phenolic compounds (Broeckling et al., 2005). However, yeast molecules do not stimulate phenolic production necessarily by stimulating PAL activity, as synthesis of PAL was reduced in *Malus domestica* cultures after yeast elicitation, although accumulation of p-coumaric and chlorogenic acid at 2.5 and, respectively, 5.1 fold compared to untreated cells was recorded (Cai et al., 2014).

Ascorbic acid is an important primary metabolite of the plant that functions as an antioxidant, enzyme cofactor and cellular signalling modulator in a wide range of essential physiological processes, including cell wall biosynthesis, secondary metabolites and phytohormones, stress resistance, photo protection, cell division and growth. It is a small water-soluble antioxidant molecule that acts as a primary substrate in the enzymatic detoxification cycle of hydrogen peroxide (Randhir et al., 2007).

Limón and his collaborators have observed in the research that ascorbic acid have a positive influence on the germination percentage of bean seeds. Thus, on day

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4 after the experiment, the germination percentage reached 60% for folic acid treated beans and 81% for ascorbic acid treated. On day 6 there was an increase of 98% for folic acid-treated seeds and 89% for ascorbic acid-treated seeds. On day 8 from cultivation, a 94% germination percentage was observed for folic acid-stimulated seeds and 90% for those stimulated with ascorbic acid. In the case of control germs, a germination percentage of 65% was observed on day 4 after experimentation, followed by a rapid increase of up to 88% on day 6 and 90% on day 8 from the time of cultivation (Limón et al. 2013).

In lentil sprouts, 500 µM ascorbic acid increased germination rates, especially up to 6 days of cultivation, as well as the total phenolic content and antioxidant activity (Penas et al., 2015).

The consumption of microgreens can be associated with benefits specific to functional foods. Microgreens, as plants in an initial developmental stage, contain high amounts of protective compounds, such as phenolics. In the same time, microgreens are normally consumed unprocessed, therefore retaining the entire chemical composition unaltered. Phenolic compounds present health related benefits, such as prevention of molecule alteration by free radicals as a result of their antioxidant capacity. Therefore, phenolic substances are related to prevention of conditions such as diabetes (Martins et al., 2011). Also, phenolic compounds are considered to exert anti-atherogenic, anti-inflammatory, anti-allergenic, antimicrobial, cardioprotective and vasodilatory activities (Shahidi et al., 2015). Production of foods with increased contents of such compounds may be obtained by genetic engineering, approach that may induce unexpected effects, due to the high complexity of metabolic pathways, while also not favoured due to consumer attitude (Boudet, 2007). The more appealing approach is to use elicitors of metabolic pathways, of physical or chemical origin, that may offer significant improvements over the untreated material (Gawlik-Dziki et al., 2013).

Cereals are a good source of phenolics, presenting antioxidant activity higher or comparable to other foods such as plums, strawberries or oranges. Among them, rye contains high amounts of phenolic acids, especially ferulic and p-coumaric acid (Dykes et al., 2007). Germination of seeds leads to transformations of deposited substances, which the consumer may benefit, such as increased polyunsaturated fat content, synthesis of mono and oligosaccharides from polysaccharides, transformation of fats into free fatty acids, transformation of proteins into peptides and aminoacids, synthesis of antioxidant enzymes (Marton et al., 2010). As such, superior quality foods may be obtained, and sprouts may be considered functional foods (Chon, 2013). In the same time, improving the quality of foods such as sprouts by using low cost elicitors, such as spent yeast, may offer viable alternatives to more expensive food supplements while retaining attributes such as natural and affordable.

CONCLUSION:

Treatment of rye seeds with spent brewery yeast or ascorbic acid may offer improved yield (length and mass) and biochemical characteristics of rye sprouts. Considering that this is the first report on stimulating rye sprouts with spent brewery yeast, such substrate may prove usable for obtaining improved sprouts of other species, for which effects of treatment, timing and concentration should be analysed.

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