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In vitro responses of raspberry plantlets to the culture media enriched with some plant sprout powders

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ABSTRACT

The growth and morphogenesis responses of cultured cell and plant tissues can be improved by addition of small amounts of some organic materials like plant sprout powders (PSP). A preliminary experiment was undertaken to examine the response of raspberry plantlets to media enriched with plant sprout powders derived from wheat, vetch and alfalfa plant species. The Murashige and Skoog (MS) medium supplemented with IBA (2.0 mg/l) and activated charcoal (200 mg/l) was exploited as optimized shoot proliferation for raspberry. Seeds of wheat, vetch or alfalfa were subjected to germination and when the rootlets attained 1 cm long, the sprouts were dried and ground to provide a homogenous powder. The shoot proliferation medium already optimized for raspberry was supplemented with 1.0 g/l of each wheat, vetch, or alfalfa sprout powders. The double-node cuttings of in vitro grown raspberries were inoculated on these media. Therefore, the growth responses of raspberries were measured in media with or without PSP. The results revealed the positive response of raspberry plantlets to the medium containing vetch sprout powder and most of the growth parameters including number of shoots, leaves, roots, and plantlet length were improved. Besides obvious apparent quality of in vitro cultures and better growth performance, the plantlets grown on media enriched with vetch sprout powder showed highest leaf number (6.41) and longer internodal length (1.89 mm). Therefore, the obtained data encourage the utilization of PSP in plant tissue culture media.

***Keywords*:** *In vitro* culture, plant sprout powder, raspberry**,** tissue culture

INTRODUCTION

Raspberry (*Rubus idaeus* L.) is a nutritious plant species that has been produced commercially in Europe and the US since the early 19th century and the *Rubus* genus is widely spread across the temperate zones (Pergolotti *et al*., 2023). The European varieties of red raspberry were introduced to the US and were crossed with the native species. Recently, interests have been provoked to raspberry because of its high nutritional value and its high contents of vitamin A, C, fibers, and antioxidant compounds (Barney *et al*., 2007). Since raspberry is perennial, its improved cultivars and elites should be preserved for both field planting and tissue culture. Shoot multiplication is extensively employed to propagate small fruits *in vitro* because it can produce disease-free plants in sterile conditions with a high propagation rate. Given the highest genetic diversity of raspberry cultivars that require a diverse set of nutrients, micropropagation of raspberry is laborious (Zawadzka and Orlikowska, 2006; Wu *et al*., 2009). So, a critical factor for viable propagation of this plant species is to have an optimal culture medium.

The growth response and *in vitro* morphogenesis of cultured plant tissues can be significantly improved by addition of small amounts of certain organic elements. Besides a natural source of carbon, organic additives may contain different natural vitamins, proteins, fiber, phenols, and also plant hormones (Khorsha *et al*., 2016). The viability of the culture of a plant's cells, tissue, or organ depends on various parameters, the most effective ones being the selection of nutritional compounds and growth regulators. A plant culture medium contains obligatory and arbitrary compound requirements, and such medium varies with cultivar, species, and explants; however, a culture

medium should contain all essential nutrients required by the plants at their optimal levels. In this sense, high production cost, which is partially associated with these compounds, is a disadvantage of plant tissue culture techniques.

High prices of chemicals have limited their extensive use in developing countries. So, the interest for the use of available highly nutritional compounds in tissue culture media is increasing. Researchers have focused on the effects of applying yeast and plant extracts in tissue culture media. Coconut milk stimulates cell division and is used as an additive in many laboratories (Hamdeni *et al*., 2022). There are numerous reports on health benefits as well as nutritional facts of plant sprouts. The nutritional and medicinal properties of sprouts are derived from their remarkable vitamin, minerals, and organic compounds content. They contain a significant amount of protein and dietary fiber as well as vitamin K, folate, pantothenic acid, niacin, thiamin, vitamin C, A and riboflavin. In case of minerals, they contain Mn, Cu, Zn, Mg, Ca and Fe. Many of these nutrient compounds increase dramatically as the sprout continues to develop. Hence, the present study aimed to explore the feasibility of application of different plant sprout powders (PSP), as an inexpensive and accessible organic source to enrich the plant tissue culture media in Raspberry.

MATERIALS AND METHODS

The present study was carried out in the plant tissue culture laboratory, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran based on a completely randomized design with four replications. Initially, a series of experiments was conducted to standardize the shoot proliferation medium of raspberry plantlets (Alizadeh *et al*., 2016). The MS (Murashige and Skoog, 1962) basal medium containing 2 mg/l IBA and 200 mg/l activated charcoal was found to be the optimum culture medium for shoot proliferation of raspberry. So, this standardized culture medium was supplemented with different doses of already ground, oven-dried wheat, vetch, or alfalfa sprout powders. In overall, five culture media were evaluated, namely: the standardized proliferation medium optimized for *in vitro* shoot proliferation of raspberry (MS), media supplemented with wheat (WSP), vetch (VSP) and alfalfa (ASP) sprout powders. Furthermore, to discriminate the role of MS ingredients and PSP effects, a medium containing only PSP but lacking MS salts was used as control (C).

To prepare PSP, a bulk seeds of wheat (*Triticum aestivum)*, vetch (*Vicia sativa*) and alfalfa (*Medicago sativa)* were prepared. The broken and defective seeds were excluded, and the healthy seeds were selected for germination. The seeds were thoroughly washed and were then soaked in water for 12 hours to swell. Then, the extra water was removed, and the seeds were wrapped between two pieces of wet cloths and were left at room temperature to germinate (5-7 days). When the rootlets were 1 cm long, they were taken away from the cloth wrappers and the sprouts were oven-dried at 55°C. This dry mass was, then, ground to provide a homogenous powder (PSP). The PSP were refrigerated in glass bottles prior to utilization in tissue culture media. The optimized MS medium for raspberry proliferation was supplemented with 1.0 g/l of each wheat, vetch, or alfalfa sprout powders. The double-node cuttings of *in vitro* grown raspberries that had been already propagated by consecutive reproduction in previous stages were prepared and inoculated on these media. Therefore, the growth responses of raspberries were measured in media with or without PSP.

Four weeks after inoculation, the samples were subjected to the measurement of fresh and dry weights, as well as some growth parameters such as visual assessment (apparent quality of the cultures (Fig.1)), chlorophyll content, plantlet length, the number of shoots, roots, and leaves, and internode length. Visual assessment of the cultures was performed as scoring ranged from 1-5 based on visual performance. The score 1 stands for least and 5 for highest visual status. The scoring was undertaken for at least 5 cultures and the average was used for analysis. Leaf area was measured with the Image J software package. The internode length and root length were measured with a caliper and ruler respectively. Also, chlorophyll content was measured with the method described in Barnes *et al*. (1992). In their method, the dimethyl sulphoxide (DMSO) is utilized as solvent to extract pigments. The 500 mg leaf tissues were collected and cut into small pieces. These were poured into the test tubes,

and then 5 ml DMSO solution was added to the tubes, then it was placed in an oven at a temperature of 75 degrees Celsius for three hours. Then 1 ml of the solution was transferred to another tube and 2 ml fresh DMSO was added to the samples. Then, the optical absorption of the solution was read using a spectrophotometer at wavelengths of 480, 510, 645 and 663 nm. Pure DMSO was used as a blank. The amount of chlorophyll a, b, total chlorophyll and carotenoid was calculated using the related formula.

Furthermore, the *in vitro* grown leaves were scanned (Fig. 2) and their area were measured by J Image software. The data were analyzed by the SAS software package and the mean data were compared by LSD test at the $p < 0.01$ and $p < 0.05$ levels.

RESULTS AND DISCUSSION

The growth response and *in vitro* morphogenesis of cultured plant cells and tissues can be improved by addition of small amounts of certain organic elements. The PSP is a natural source of carbon, and it may contain several vitamins, phenols, fiber, hormones and also proteins. Hence, their addition to the media may have a positive role (Alizadeh *et al.,* 2016). The supplementation of PSP had desirable impacts on growth traits of raspberries under *in vitro* culture conditions. The analysis of variance showed that the PSP treatments had significant impacts on apparent quality, chlorophyll content, root number and plantlets fresh or dry weights at the $p < 0.01$ level and on leaf number, internode length, and leaf area at the $p < 0.05$ level. However, PSP had no significant effects on number of raspberry shoots (Table 1). In a general vision, it can be observed that the plantlets grown on media supplemented with PSP have better visual quality (Fig.1), more broad and attractive leaves (Fig. 2). The VSP enriched media gave more vigorous cultures as compared to other treatments.

The commercial tissue culture technique is influenced by several factors including the right selection of plant species, physical environment, and chemicals for the culture medium (Puchooa and Ramburn, 2004). The concentration and type of carbon sources added to the culture medium are effective in the success of tissue culture. Sugars are essential as a source of energy and also, to hold the osmotic potential of the culture medium (Lipavská and Konrádová, 2004). Carbohydrates have various functions in tissue culture, including energy supply to *in vitro* plants, especially at the early stages of the tissue culture cycle when the photosynthesis rate is still low, cell growth when the cells are exposed to radiation that is outside of photosynthetically active range, and the generation of osmotic pressure; it also has certain morphogenetic impacts in some cases (Al-Khateeb, 2008; Anwar *et al*., 2005). Carbon sources can have simple and/or complex sugars (Akter *et al*., 2007). Most cultures rely on a carbohydrate source as long as they are prepared for adaptation.

Chlorophyll and carotenoid are two major photosynthetic pigments. The frequency of these pigments would be critical especially when the tissue culture raised plantlets are taken out to *ex vitro* (soil) conditions. The leaves with high density pigments may increase the survival percentage during hardening stage or *ex-vitro* transfer. The wider leaves with high chlorophyll content contribute to higher photosynthetic rate in these plantlets. Such alterations mitigate transplanting shock during hardening stage. The application of seaweed extract as growth stimulator to some plants (Fornes *et al*., 2002; Vernieri *et al*., 2005) enhanced chlorophyll content and photosynthesis rate of the leaves. Khorsha *et al.* (2016) reported that the application of apricot gum as an organic compound to the culture medium of *Stevia*, a medicinal plant, increased chlorophyll content versus control 28 days after inoculation. In the present study (Table 2), the media supplemented with PSP had chlorophyll content comparable to MS media. However, the highest chlorophyll content (5.78 mg/g FW) was recorded in plantlets grown on MS medium but it was not significantly different with PSP enriched media. This observation was corroborating with Khorsha *et al*. (2016).

There are numerous reports on the use of inexpensive and convenient organic resources for the rooting of plants in the tissue culture medium, such as for beet molasses, sugarcane juice (Buah *et al*., 2011), and date palm syrup (Al-Khateeb, 2008). The application of apricot gum to the grapevine and Stevia culture media increased the number and length of the roots (Khorsha *et al.,* 2016). The rooting in raspberry explants was

Table 1: Analysis of variance for the effect of plant sprout powder (PSP) on the *in vitro* performance of raspberry.

Sources of variations	df	Visual assessment	Chlorophyll	Shoot no.	Root no.	Leaf no.	Fresh weight	Dry weight	Root length	Plantlet length	Internode length	Leaf area
Treatment	4	0.4^{**}	4.52^{**}	0.78^{ns}	1.53^{**}	5.14^{*}	0.41^{**}	0.04^{**}	3.87^{**}	0.24^{*}	0.17^{*}	0.19^{*}
Error	10	0.05	0.31	0.28	0.006	1.03	0.01	0.001	0.04	0.06	0.03	0.03
CV		13.26	11.73	27.06	16.41	21.69	12.51	10.26	26.9	14.5	11.03	24.9

**: significance at the $p < 0.01$ level; *: significance at the $p < 0.05$ level; ns: non-significance.

observed only in MS and VSP supplemented media. The highest number of roots (1.52) was recorded in VSP, while the highest root length (2.48 cm) was realized in MS treatment (Table 3). The existence of long roots may not be essential for plantlets under *in vitro* conditions because they have easy access to water and nutrients under *in vitro* conditions. Even explants with no roots are capable of nutrient absorption from medium. However, longer roots with several small and diffuse hairy roots would be significant for *ex vitro* transfer phase (Alizadeh *et al*., 2010). Therefore, they provide the plantlets higher area for the uptake of water and nutrients and reduced plantlet loss during acclimation.

Different organic additives and sugars were applied as carbon sources to the growth and propagation of PLBs of *Dendrobium noble* plants, which were supplemented with banana homogenate, tomato homogenate, and coconut milk at different rates. The results showed that banana and tomato homogenates were effective in the proliferation of orchids and that the coconut milk was shown to be the best organic additive for the proliferation of PLBs so that it increased fresh weight by four times in only 4 weeks when compared to the initial weight. The highest growth in PLBs was recorded for glucose, fructose, and sucrose, 0.94 ± 0.55, 9.1 ± 0.82, and 6.51 ± 0.52 g, respectively. Galactose, mannitol, and sorbitol were desirable for increasing the growth of PLBs. They introduced coconut milk as the best organic additive and glucose as the best carbohydrate source for the propagation of orchid PLBs. Some other organic additives such as coconut milk, banana homogenate, potato pulp and juice, honey, date pulp syrup, corn pulp, papaya pulp, and beef extract have also been utilized in plant tissue culture studies (Murdad *et al*., 2010). Organic additives were reported to contribute to the production of PLBs, shoots and leaves (Akter *et al*., 2007), increased the size of somatic embryos (Al-Khateeb, 2008), and help the growth and develop of seeds and regeneration (Tawaro *et al*., 2008). In an experiment undertaken by Puchooa and Ramburn (2004), it was found that the increase in fresh and dry weights of explants was lower in culture media containing carrot juice as compared to media supplemented with cytokinin and auxin. However, with the increase in carrot juice concentration, the fresh and

Table 2: The effect of plant sprout powder (PSP) on some *in vitro* parameters of raspberries

Treatments	Visual assessment	Chlorophyll mg/g FW	Leaf no.	Leaf area cm^2	Shoot no.	Shoot length cm	Internode length(cm)
MS*	2.00a	5.78a	4.00b	1.12a	1.47b	2.04a	1.51bc
VSP	2.04a	5.07a	6.41a	0.82a	2.64a	1.90a	1.89a
ASP	1.85a	5.18a	5.24ab	0.73ab	2.27ab	1.80a	1.74ab
WSP	1.90a	5.21a	4.90ab	0.81a	2.06ab	1.74a	1.61ab
C	1.15b	2.64b	2.94c	0.41b	1.47b	1.29b	1.26c

*MS: Murashige and Skoog medium; VSP, ASP, WSP: Vetch, Alfalfa and wheat sprout powder respectively; C: control. The means indicated by the same letter in each column are not statistically different ($P < 0.01$).

Table 3: Mean comparison for the effect of plant sprout powders on raspberry plantlets

Treatments	Root length(cm)	Root no.	Fresh weight (g)	Dry weight (g)
MS*	2.48a	1.00ab	0.67b	0.31b
VSP	1.45b	1.52a	1.24a	0.47a
ASP	0.00c	0.00c	0.59b	0.26b
WSP	0.00c	0.00c	1.14a	0.43a
C	0.00c	0.00c	0.37c	0.17c

*MS: Murashige and Skoog medium; VSP, ASP, WSP: Vetch, Alfalfa and wheat sprout powder respectively; C: control. The means indicated by the same letter in each column are not statistically different ($P < 0.01$).

dry weights were also increased. In our experiment, we found higher fresh and dry weights of *in vitro* grown raspberry plantlets in media supplemented with either wheat or vetch sprout powders (Table 3).

The usefulness of organic additives in plant tissue culture media was also highlighted in some other articles. For example, the effect of organic additives on the proliferation of orchids in half-strength MS culture medium enriched with local banana homogenate, tomato homogenate, and immature coconut milk showed that the type and concentration of organic additives influenced the proliferation response of PLBs (Nambiar *et al*., 2012). The addition of organic substances to culture media not only acts as an organic carbon source but they also contain natural vitamins, phenols, fibers, hormones, and proteins (Gnasekaran *et al*., 2010).

Different basal media are commercially exploited for tissue culture of plants and they have diverse effects on the growth and proliferation of plants depending on their macro and microelement levels. In addition to standard compounds, organic acids and a wide range of natural extracts are also applied randomly in the culture of specific species. Whenever the compounds supplemented to these basal media did not have the appropriate results, the researchers used some other compounds such as coconut milk, malt extract, tomato juice, yeast extract, or orange juice, which gave them optimal results (Molnár *et al*., 2011). Singh and Kaur (2011) revealed that the application of malt extract did not cause significant differences in proliferation percentage, but they obtained the longest branches as compared to the plants exposed to cytokinin treatments.

Khorsha *et al.* (2016) reported that the number of shoots and intermodal length of grapevines plantlets were influenced in optimized medium enriched with apricot gum. They observed highest number of micro-shoots and longer internodes in media supplemented with 4 g/l apricot gum. In another report by Khorsha (2014) the highest number of shoots in stevia plantlets was observed in medium enriched with apricot gum at the rate of 6 g/l. The raspberry tissue culture media enriched with PSP had considerable influence on shoot proliferation (Table 2). The highest number of shoots (2.64) and long internodes (1.89 cm) were obtained in VSP supplemented media. These results are consistent with Khorsha *et al.* (2016).

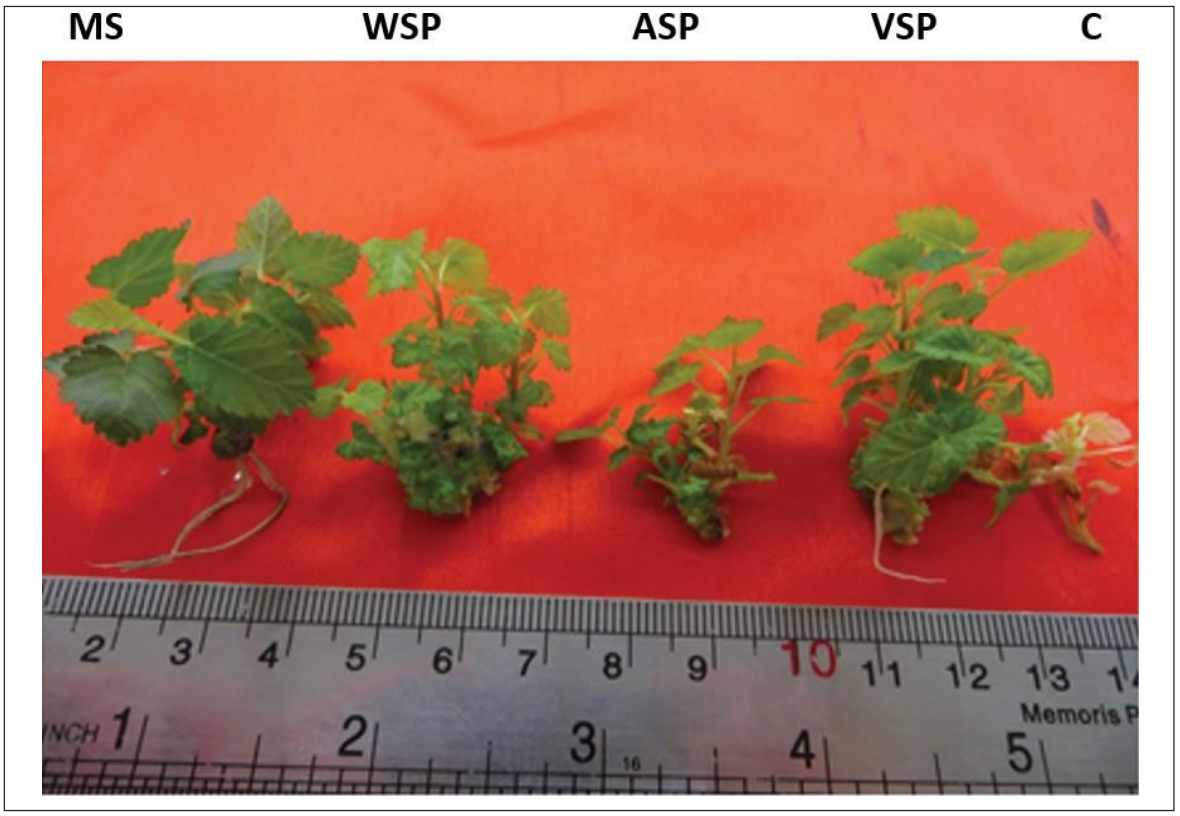

Fig. 1 : The morphology of *in vitro* grown raspberry plantlets as affected by media enriched with plant sprout powders. MS: Murashige and Skoog medium; VSP, ASP, WSP: Vetch, Alfalfa and wheat sprout powder respectively; C: control.

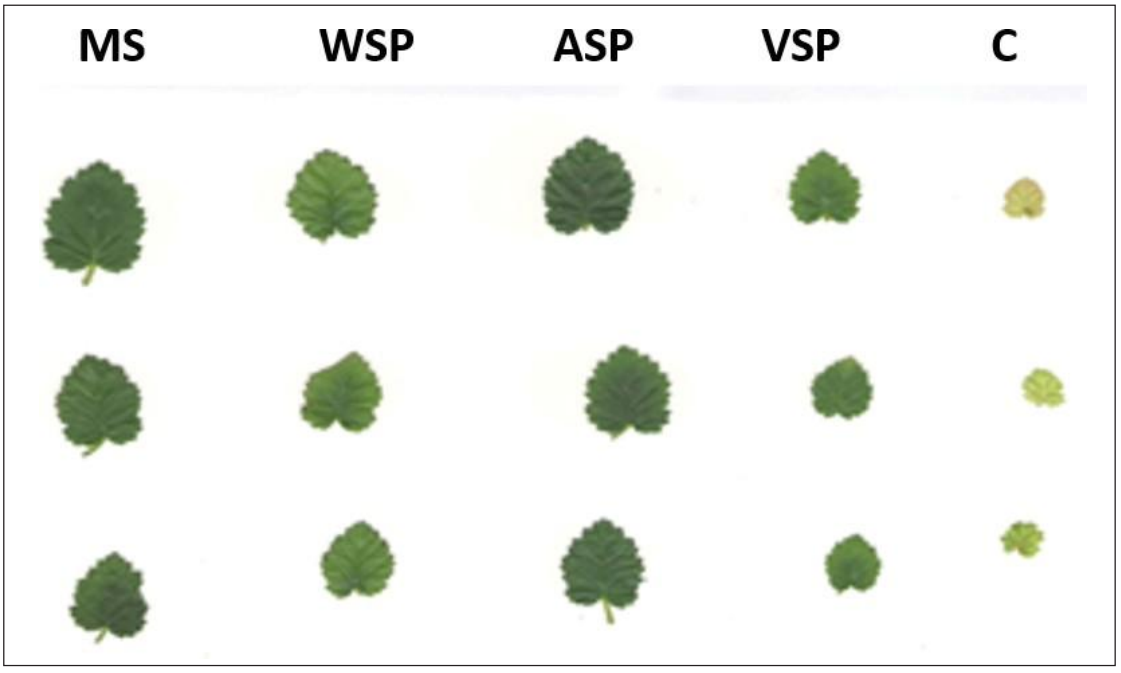

Fig. 2: Samples of *in vitro* raspberry leaves scanned with a scanner. MS: Murashige and Skoog medium; VSP, ASP, WSP: Vetch, Alfalfa and wheat sprout powder respectively; C: control.

CONCLUSION

In conclusion it can be stated that, PSP have nutritious constituents that can be used as a substitute or supplement to tissue culture media. Furthermore, addition of VSP may improve the growth parameters of *in vitro* raspberry plantlets and its utilization in tissue culture media may be encouraged. However, though demonstration of positive effects of PSP-enriched media in our study, recommendation for application of such ingredients as a constant part of media culture or in commercial laboratories, needs to further complementary experiments. Furthermore, it is recommended to examine the PSP derived from other seeds such as barley, pea and corn to find out the rate of *in vitro* growth and proliferation of plants.

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