A MEDIUM CONTAINING COMMERCIAL FOLIAR FERTILIZER AND SOME ORGANIC ADDITIVES COULD SUBSTITUTE MS MEDIUM FOR *IN VITRO* GROWTH OF *DENDROBIUM* HYBRID SEEDLINGS

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ABSTRACT

In an attempt to obtain a cost-effective and a simpler way of growing *Dendrobium* seedling *in vitro*. This study was conducted to investigate the effects of basal media and organic additives on *in vitro* growth of *Dendrobium* seedlings. There were two factors treatment. The basal media used were first media containing half strength Murashige and Skoog (½ MS medium) and second media containing foliar fertililizer 32:10:10 and trace elements (FT medium). The organic additives were tomato, potato, mungbean sprout juice and banana homogenate. The experiment was conducted in a completely randomized design with three replicates, 5 culture vessels per replicate and 4 seedlings ca. 1.5 cm per vessel. The experiment resulted that FT medium had significantly higher growth than ½ MS medium as indicated by seedling fresh weight. The media containing tomato juice led to the highest growth of seedling as shown by seedling fresh weight and seedling height. Since FT medium caused better growth than ½ MS medium, the FT medium could be used as a substitute for ½ MS medium for growing *Dendrobium* hybrid seedlings.The seedlings were successfully acclimatized in a shaded green house environment with 100% survival rate.

Key Words: Orchids, cost-effective, propagation, tomato juice, foliar fertilizer.

INTRODUCTION

Indonesia is considered as one of the orchid biodiversity centers, home of approximately 5000 species of some 25000 species of orchids in the world (Pusat Data dan Sistem Informasi Pertanian, 2015). Unfortunately, this richness in orchid biodiversity has not been optimally transformed into economic advantage. Data from the year of 2000 to 2014 reveals that while the production of orchid increased, the export in the form of plants and seedlings sharply decreased from 673,115 to 52,651 kg, in which the export of plants decreased from 338,556 to 268 kg and that of seedlings decreased from 334,559 to 52,383 kg (Pusat Data dan Sistem Informasi Pertanian, 2015). In the period of 2010-2011 there was no export of seedlings, while during 2012-2014 the export was relatively

stagnant, the figure being from 52,383 to 54,973 kg (Pusat Data dan Sistem Informasi Pertanian, 2015). These data indicate that Indonesia is having problem with the production of orchid seedlings, which is paradoxal since this country is one of the centers of orchid biodiversity.

One way of producing orchid seedlings is by germinating seeds to form protocorms *in vitro* and then develop them into seedlings. One of the most frequent constraints for orchid grower is to produce a large number of high quality seedlings with reasonable price. To produce high quality seedlings by means of tissue culture is considered costly. Standard laboratory medium such as Murashige and Skoog (1962) is still considered costly for many orchid seedling producers. Some researches has been conducted to test the possibility of using commercial fertilizers supplemented with organic additives to substitutes standard plant tissie culture medium. The commercial fertilizers are expected to provide mineral nutrition, while the organic additives are expected to serve as inexpesive and easy-to-find sources of amino acids, peptides, vitamins and plant growth regulators in various concentrations. These natural organic additives were reported to have beneficial effects for *in vitro* plant cultures (Thorpe *et al*., 2008).

In an attempt to find a more cost-effective medium, this study was conducted to evaluate effects of basal medium containing commercial foliar fertilizer 32:10:10 and trace elements compared to ½ MS as basal media and to evaluate effects of some organic additives, those were tomato, potato, mungbean sprout juices and banana homogenate, on growth of *Dendrobium* seedlings *in vitro*.

MATERIALS AND METHODS

Plant Materials.

Aseptic seedlings of *Dendrobium* hybrids (± 1.0 cm) with two open leaves were used as starting plant materials. These seedlings were obtained from 4-month old germinated seeds resulted from hybridization of two *Dendrobium* hybrid-parents, collection of the Plant Science Laboratory, The University of Lampung.

Media and Culture Condition

The basal media used were first Murashige and Skoog (1/2 MS medium) and the second containing 2 g L^{-1}foliar fertilizer NPK 32:10:10 and trace elements (FT medium). Each basal medium was supplemented with different kinds of organic additives (tomato, potato and mungbean sprout juices and ripe banana homogenate). The foliar fertilizer consists of 32% N, 10% available phosphoric acid (P_2O_5), 10% soluble potash (K_2O), 0.05% Ca, 0.1% chelated magnesium (Mg), 0.2% sulphur compounds (S), 0.02% boron (B) 0.05% chelated copper (Cu), 0.1% chelated iron, 0.05% chelated manganese (Mn), 0.05% chelated zink (Zn) and 0.0005% molibdenum (Mo). All media formulations were supplemented with 20 g L^{-1} sucrose and 150 ml L^{-1} coconut water. The pH

of media were adjusted to 5.5 prior to being solidified with 7 g L^{-1} agar. The media were boiled and dispensed into 350-ml culture bottles (30 ml/bottle). All of the bottles containing media were capped with plastic sheets and sterilized for 15 minutes using autoclave (Tommy, Japan) at 121^0C and 1.5 kg cm^{-2} pressure. Aseptic *Dendrobium* seedlings were cultured on the media and then maintained in a culture room with illumination of cool white fluorescent light of approximately 2000 lux and a 16-h photoperiod at 26 ± 2^0C.

Experimental Design and Data Analyses

Treatments were factorially arranged, first factor being basal media (1/2MS and FT) and the second one being organic additives (tomato, potato and mungbean sprout juices and ripe banana homogenate). The experiment were arranged in a completely randomized design with three replications. Each replication consisted of 5 culture bottles, and each culture bottle contained 4 seedlings. After three months in culture, seedling height, leaf number, root number, root length, and fresh weight were recorded. All data were subjected to analysis of variance and mean separation was carried out using least significant difference (LSD). Seedlings of 5-8 cm were taken out from culture bottles, washed from the sticking medium and planted on fern chips medium as community pots and acclimatized in a shaded greenhouse.

RESULTS AND DISCUSSION

Results

After three months in cultures, all seedlings showed normal growth and did not show any symptoms of nutrient defficiency. The seedlings had four or more leaves and five or more roots (Figure 3,4, and 6) They looked healthy, their sizes being variables depending on the treatments. The basal media significantly affected all of the variables, except root length. Organic additives significantly affected fresh weight, seedling height and root length but did not significantly affect leaf number and root number. The basal media and organic additives showed significant interaction in affecting all of the variables, except root length.

After three months in cultures, seedling height increased three to almost seven folds compared to the initial size (±1 cm). FT medium led to better seedling growth than ½ MS medium at all of organic additives supplemented, as indicated by seedling fresh weight. (Figure 1). FT medium resulted in the same or more leaves than ½ MS medium (Figure 3) and more roots at all organic additives except banana homogenates (Figure 4). The basal media did not significantly affect root length.

Among treatments with organic additives, a treatment with tomato extract resulted in highest growth as indicated by seedling fresh weight and seedling height whether in combination with ½ MS or FT medium(Figure 1 and 2). In each basal

medium, potato extract was the second best after tomato extract for growth promotion, as indicated by seedling fresh weigth and seedling height, followed by either mungbean sprout extract or banana homogenate (Figure 1 and 2). As far as leaf number was concerned, at each medium treatment tomato extract led to more or less the same leaf number compared to other organic additives, except when compared to mungbean sprout extract. A treatment with mungbean sprout extract resulted in highest leaf number (Figure 3). Compared to other organic additives, banana homogenate led to the highest root number on ½ MS medium and the lowest root number on FT medium (Figure 4). The longest root was achieved on medium supplemented with potato extract, followed by tomato extract, mungbean extract and banana homogenate (Figure 4).

Representative appearance of *Dendrobium* seedlings in both basal media and all additives tested were shown in Figure 6. Even though all seedlings were healthy and produced adequate number of roots (more than 4 roots) for acclimatization, seedlings grown in FT medium supplemented with various organic additives (Figure 6-e,f,g,h) were generally larger, sturdier, and more vigorous with larger stem diameters, compared to those grown on ½ MS medium (Figure 6-a,b,c,d).

Discussion

In this experiment, the possibility of using FT medium containing commercial fertilizer NPK 32:10:10 and trace elements to substitute MS medium in orchid tissue culture was explored with objective of getting more cost-effective production of *Dendrobium* seedlings. The experiment showed that the FT medium resulted in the same or better seedling growth compared to the ½ MS medium, depending on organic additives added. This results indicate that even though mineral nutrients in FT medium was not as complete as those in ½ MS medium, the FT medium supplemented with organic additivescould provide essential macro and micronutrients required for growth of *Dendrobium*seedlings. The promoting effects of FT medium suplemented with organic additives was probably due to balanced supply of nutrients from the fertilizer itself and from the organic additives in the form of simple ionic minerals or more readily consumed forms, i.e. organic compounds. This results indicate that the FT medium could be used as a substitute for a commonly used ½ MS medium for growing seedling of *Dendrobium* orchids *in vitro*. The same suggestion was also put forward by Yulika (2007) based on her finding that medium containing commercial fertilizer resulted in better gowth of *Phalaenopsis* seedlings than½ MS medium. Similar results were reported by Winarto *et al*. (2015) in tissue culture of *Dendrobium*, showing that medium containing a commercial fertilizer resulted in the same or better

growth of protocorms and seedlings compared to MS medium, as indicated by percent PLBs germination, number of germinated PLBs, number of leaves per germinated PLBs, leaf length, and root length. In their experiment, the media were supplemented with coconut water.

Various organic additives has been reported to promote germination and/or growth of some orchids *in vitro* (Muthukrishnan *et al*., 2013; Kaur and Bhutani, 2012; Aktar *et al*., 2008; Xuan, 2015; Gnasekaran *et al*., 2012; Shadang *et al.*, 2007; Jawan *et al.*, 2010; Parthibhan *et al*., 2015; Murdad *et al*., 2010; David *et al*., 2015). The promoting effects have probably been attributable to organic nutrients and growth factors contained in the organic additives. In our experiment, all of the media contained 15 % coconut water. Therefore, treatments used in this experiment were basically intended to study effects of additional organic additives other than coconut water. Coconut water has been used in tissue culture of a number of plants and often used in tissue culture of orchids. Coconut water contains plant hormones (such as auxins and cytokinins), vitamins, various amino acids, organic acids, nucleic acids, sugar, sugar alcohols, mineral nutrients, and other unidentified chemicals (Molnar *et al*., 2011).Adding coconut water to plant tissue culture media has often become an easy way to get satisfactory growth and development without necessity to work out suitable media composition (Thorpe *et al*., 2008). Therefore, it could be assumed that without additional organic additives as treatments, the media containing coconut water should have provided nutrients and growth factors for normal growth of seedlings.

Our experiment showed that the best organic additives for promoting growth of *Dendrobium* seedling was tomato juice, as indicated by seedling height and freshweight. The tomato juice exhibited the highest growth-promoting effects whether it was added to FT medium or ½ MS medium. Similar results were reported by Muthukrishnan*et al*.(2013) that among organic additives used in their experiment (tomato juice, coconut water, potato juice), tomato juice caused the best seed germination in*Geodorum densiflorum* (Lam) Schltr., when supplemented to ½ MS medium. Gnasekaran *et al*. (2012) also reported that addition of tomato extract to basal medium resulted in better growth of protocorms of Vanda Kasem's Delight compared to papaya and potato extracts. Dwiyani *et al*. (2015) found that tomato juice supplemented to new phalaenopsis (NP) medium led to better gowth of protocorm and seedlings of *Vanda* orchids compared to coconut water.

As proviously mentioned, in our experiment, the best growth of *Dendrobium* seedlings was obtained when the medium was supplemented with tomato juice, followed by potato extract, mungbean sprouts extract, and banana homogenate. This result appeared to be related to different chemical composition of the additives.

Ripe tomato fruitshas been reported to containvarious beneficial nutrients per 180 g serving size, i.e., 24.66 mgvitamin C, 7.20 mcg biotin, 14.22 mcg vitamin K, 74.97 mcg vitamin A, 0.14 mg vitamin B6 (pyridoxine), 0.03 mg vitamin B2 (riboflavin), 1.07 mg vitamin B3 (nicotinic acid), 27 mcg folic acid, 0.07 mg vitamin B1 (thiamine), 1.25 mg niacin eq., 0.97 mg, 0.97 mg vitamin E, 0.16 mg panthothenic acid, various mineral nutrients (43.20 mg phosphorus, 426,6 mg potassium, 19.8 mg magnesium, 9 mcg molybdenum, 0.11 mg copper, 0.21 mg manganese, 0.49 mg iron, and 0.31 zinc), sugars, lycopene and β-caroten (The World Healthiest Foods, 2018). In plant tissue culture, thiamines, pyridoxine and nicotinic acid are most commonly used vitamins (Abrahamian and Kantharajah, 2011. Vitamin C, also known as ascorbic acid, is well known as an antioxidant and a cofactor for many enzymes which play various important beneficial biological roles in plants, such as in regulation of plant growth and development, cell division, cell wall metabolism and expansion, shoot apical meristem formation, root development, regulation of plant senesence, photosynthesis, stress defense, and fluoresence (Zhang, 2013). In plant tissue culture, addition of vitamin C was reported to increase shoot formation from callus of tobacco (Tomar *et al*., 2018).Sunitha (2014) reported that addition of 1 ppm vitamin C into MS medium increased the whole plant regeneration ofstem explant of *Centella asiatica*, seed explants of *Trigonella foenumgraecum* and shoot regeneration of *Santalum album* stem explants cultured *in vitro*, as well as enhanced chlorophyll contents in the *in vitro* grown plants. Thus, the superior growth effects by addition of tomato juice from 200 g ripe tomato per liter MS medium in this experiment would likely to be caused by its various vitamins and other various nutrition contents.

CONCLUSION

This research showed that a commercial foliar fertilizer (FT medium) could be used as medium for growing *Dendrobium* hybrid seedlings *in vitro*, being comparable to Murashige and Skoog (1962) (MS) medium, a standard medium for in vitro culture of plants. When supplemented with tomato extract, FT medium could significantly outperformed MS medium for supporting *Dendrobium* seedling growth *in vitro*. This finding could provide orchid farmers with simpler and cheaper alternative culture media for growing *Dendrobium* seedling *in vitro*. As far as we are concerned, this is the first report on the use of a commercial foliar fertilizer together with tomato extract that support better growth of *Dendrobium* hybrid seedlings *in vitro* than MS medium.

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FIGURES

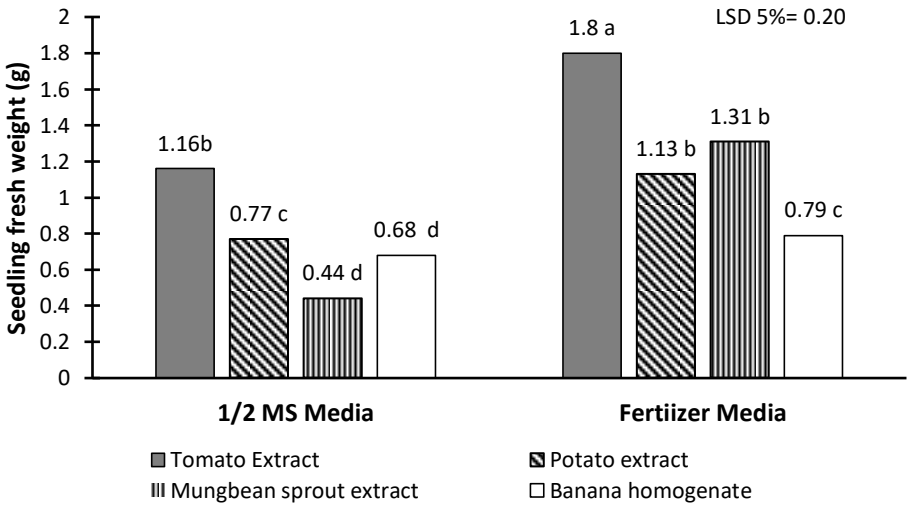

Figure 1. Effects of two basal media and various organic additives on fresh weight of *in vitro* grown *Dendrobium* hybrids seedlings after 3 months in cultures.

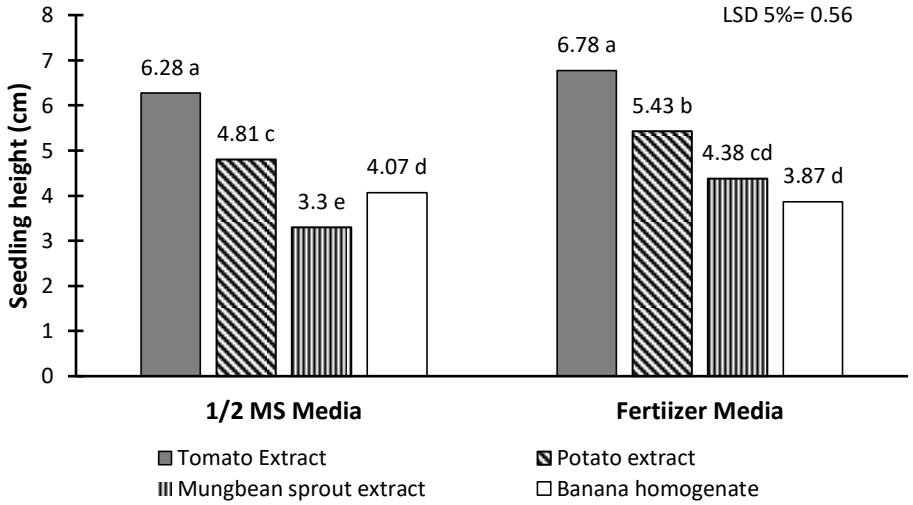

Figure 2. Effects of two basal media and various organic additives on seedling heights of in vitro grown *Dendrobium* hybrids seedlings after 3 months in cultures.

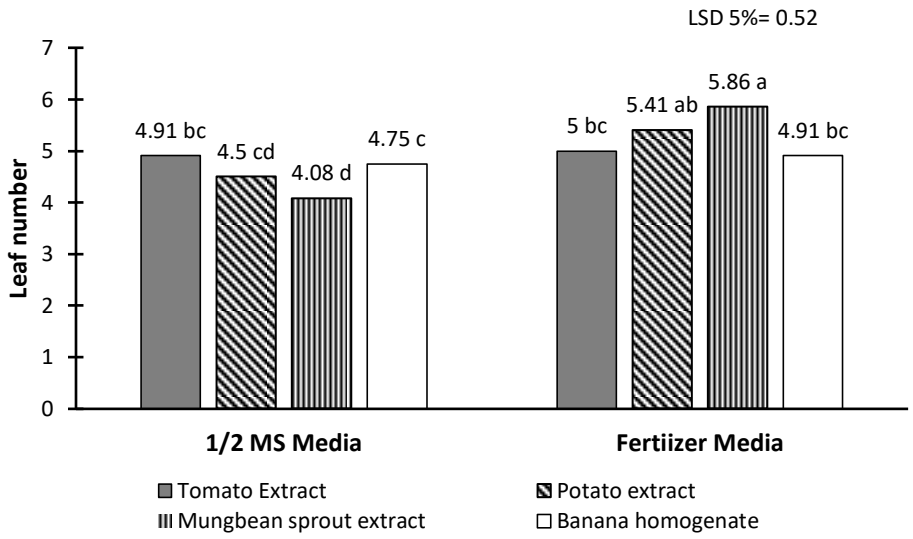

Figure 3. Effects of two basal media and various organic additives on number of leaves of *in vitro* grown *Dendrobium* hybrids seedlings after 3 months in cultures.

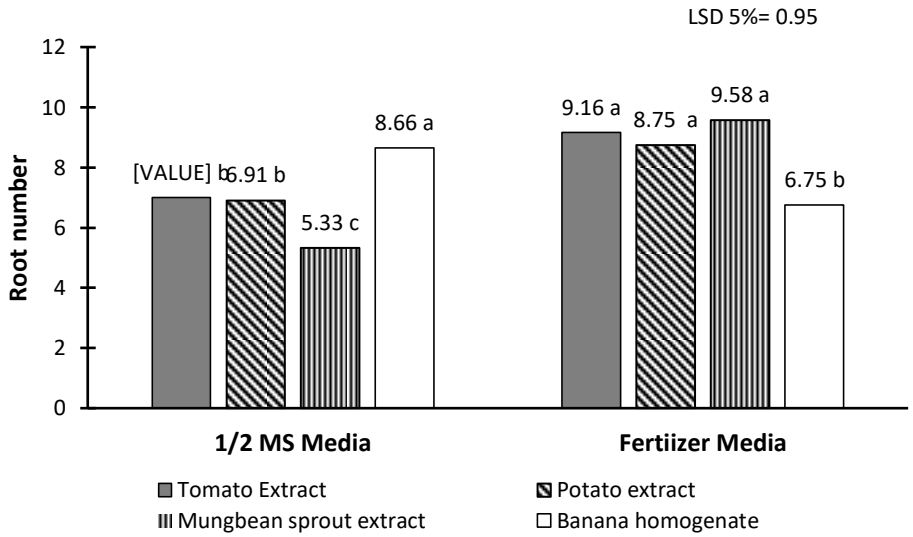

Figure 4.Effects of two basal media and various organic additives on number of roots of *in vitro* grown *Dendrobium* hybrids seedlings after 3 months in cultures.

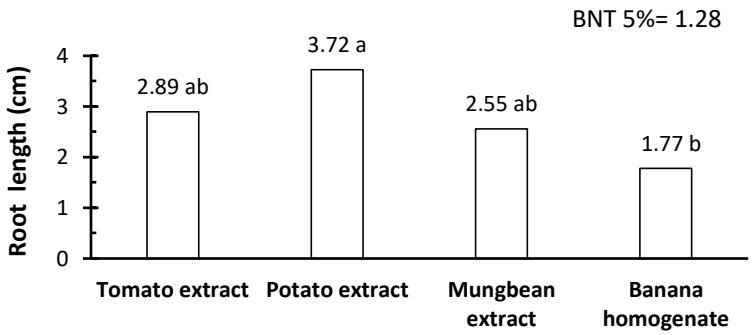

Figure 5. Effects of two basal media and various organic addenda on length of roots of *in vitro* grown *Dendrobium* hybrids seedlings after 3 months in cultures.

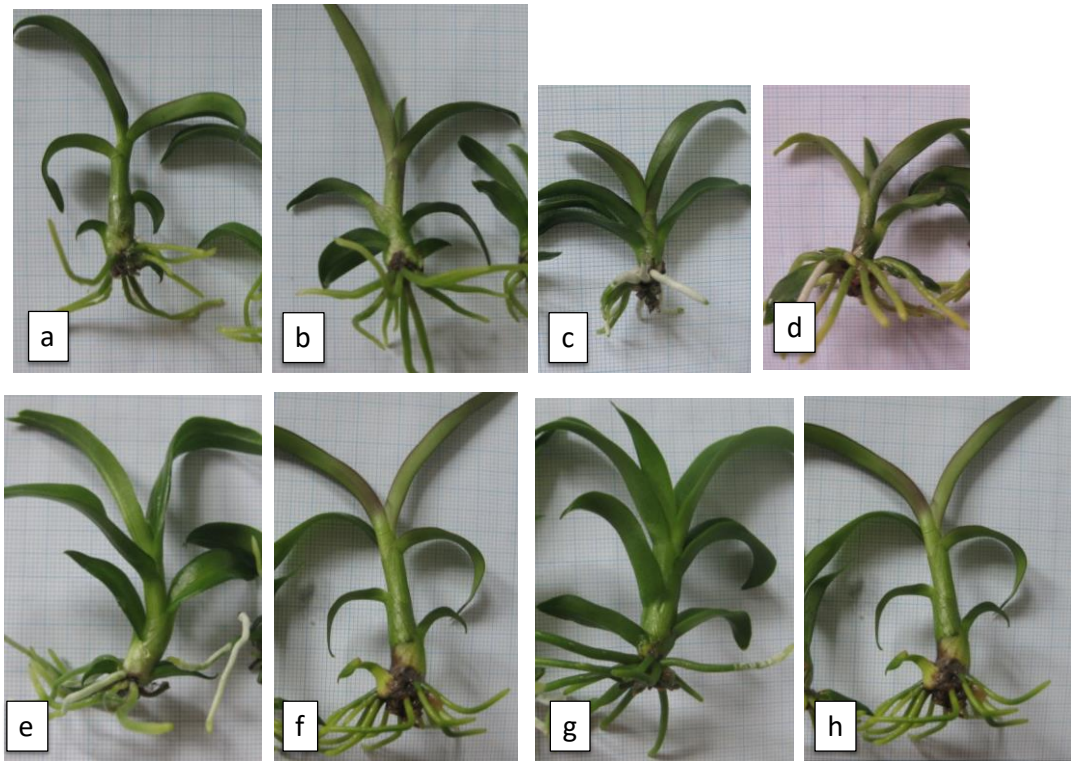

Figure 6.Representative appearence of *Dendrobium* hybrid seedlings in ½ MS (a,b,c,d,) and NPK basal media (e,f,g,h) with various additives amenden in the media, namely tomato juice (a,e), potato (b,f), mungbean sprouts (c,g) and banana homogenate (d,h) after three moths in cultures.