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Coir Pith as a Acclimatizing Medium for Micropropagated Capsicum SP

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Abstract: ***Micropropagation system was developed through an enriched culture system for Capsicum annum L. of a traditional medicinal plant and nutritionally important spice crop. Proliferation of shoots were achieved on MS medium supplemented with 13 g l-1 sucrose, 8 g l-1 agar and fortified with benzyladenine (BAP)in the rage of 0.2 -1mg/l. A higher number of shoots were observed on an enriched MS medium supplemented with 0.5mg/l BAP. Then the shoots were subjected for root induction with the concentration of 1mg/ml. For the multiple shoot induction different combination of hormones were used. The well-developed microshoots were transferred to rooting medium supplemented with IAA (1.0mg/l) for efficient rooting. The well developed plants were transferred for hardening with coir pith, because of its water holding capacity, C: N ration, pH and the porosity. The efficiency was achieved around 90% survival of the micropropagated plants.***

Keywords: Micropropagation, coir pith, shoot

1. Introduction

Coconut palm (Cocos nucifera L.) is being cultivated in many parts of the world producing around 54 billion coconuts per year from an estimated area of 12.78 million hectares (Mathew, 2004). India produces 22.3 percent of the total coconuts and is being considered as a premier coconut producer in the world. Coconut is known to play a vital role in the economy of many marginal farmers in India (Reddy and Thampan, 1988).

The coconuts are processed in industries to yield oil from dried kernels (copra). The above process generates huge quantities of husk (mesocarp) which was considered to be a waste (Mathew et al., 2000). These husks are now being utilized as a raw material for the production of coir fibre. During the extraction of coir fibre from the husk, a light weight spongy material is released. This spongy material is referred to as coir pith which accounts for 50-60 percent of the total weight of the husk. Total generation of coir pith in India is estimated to be around 0.5million tonnes per year while the world production is around 3.6 million tonnes (Pillai et al., 1981). Therefore, coir industries are facing great difficulties in the disposal of coir pith (Dan, 1993). Very often coir pith is heaped as mounds on way side. Large quantities of coir pith thus stored causes contamination of potable ground water due to the percolation of leachates containing residual phenol from these dumps (Gopal and Gupta, 2001) especially during rainy season. It also offers an ideal breeding base for rodents and insect pests (Grimwood, 1975). Coir pith is easily blown by wind due to its light weight thereby creating air pollution. In comparison to saw dust, rice husk and groundnut shell, coir pith is found to have a higher heat value (3975 kcal / kg) which is close to that of coal (Krishnan, 1990 and Sudhira and Jacob, 2000).

To overcome the problems associated with coir pith disposal, many innovative practices are being followed. It is reported by Cresswell (1992) that coir pith has higher structural stability, cation exchange capacity, water absorption ability and drainage than sphagnum peat or sedge peat. Lack of waxy cutin to repel water in coir pith has increased the wetting and rewetting ability of coir pith. The incorporation of coir pith in soil improves the physical and chemical properties of the soil (Bopaiah, 1991). It also increases the available fraction of the NPK (Clarson et al., 1983). An increase in soil moisture as well as reduction in weed growth is noticed when coir pith is used as a cover for the surface of garden soil (Bopaiah, 1991).

In certain parts of India, coir pith finds its application in making bricks (Dan, 1993) and particle-boards (Viswanathan, 1998). A combination of cow dung and coir pith at 4:1 ratio increases the biogas production (Mathew et al., 2000). Coir pith forms a good bedding material in poultry shed (Maheswarappa et al., 2000). The coir pith bed enriched with poultry litter is directly used as manure for crops such as sorghum, groundnut and sunflower (Savithri et al., 1991). Coir pith compost is successfully utilised in the cultivation of many annual crops (Gupta et al., 1986; Muthulakshmi, 1988; Ramaswami and Kothandaraman, 1991; Savithri and Khan, 1994; Taha, 2000 and Anand et al., 2002). As the bulkiness of the coir pith compost often makes very difficult to handle, it is often compressed into bricks to enable easy transportation. The soilless media are either minerals or organics. Some of the mineral media used are rockwool, foam, quartz sand, pumice, vermiculite and polystyrene. The organic media favoured by farmers are peat, coir pith, tree bark, sugarcane bagasse, saw dust, forest weeds, paddy straw, wheat straw and water weeds (Hansen et al., 1993; and Jaenicke, 1999). Among the various soilless media, peat is extensively used in field operations. Environmental concerns on the use of certain artificial materials and increased restrictions on the exploitation of peat induce a search for an alternative material (Tognoni et al., 2004).

One of the promising alternatives was coir pith (White, 2004). It is successfully used as a soilless medium to grow several foliage plants such as Anthurium (Meerow, 1995), Dieffenbachia sp. (Stamps and Evans, 1997), Dracaena sp. (Stamps and Evans, 1999) and Spathiphylum (Mak and Yeh, 2001). Coir pith compost was also tested as a

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successful pot culture medium for several ornamental and agronomic crops (Cresswell, 1992; Evans and Stamps, 1996; Meerow, 1994 and Pill and Ridley, 1998).

Chillies are the dried ripe fruits of *Capsicum annum* L. Comes under the family of Solanaceae which has about 90 genera and 2000 species (Clark, 1997). This family includes tobacco a commercial and cash crop and important vegetables like tomato, potato, brinjal etc. Chillies are widely cultivated mainly in tropic and subtropic countries like India, China, Africa and Japan. The centre of origin of chilli is said to be Mexico, Guatemala and Bulgaria. Chilli is most famous for its pleasant aromatic flavour, pungency and high colouring material. Among the spices consumed per head in India, dried chillies both as a condiment or culinary supplement and as a vegetable. Chilli is an important crop in South India and is grown for its pungent fruits, which are used both green and ripe (the latter in the dried form) to impart miniature pungency to the food (Lee et al., 2004) . The miniature fruits are widely used for its specific pungency and its significant medicinal value. It is also used medicinally, and in chutnies and pickles. The pungency is due to the higher accumulation of active principle `capsicin contained in the skin and the septa of the fruit. Tissue culture is an important technique for the rapid multiplications and for the crop improvement of medicinal and aromatic and horticulturally important crops.

The application of Modern Biotechnology to enhance the productivity of habenaro pepper requires an efficient in vitro protocol. In order to improve propagation of the commercial cultivars of this species and to meet the increasing demand for the crops, more reliable propagation approaches were need for the mass multiplication. The habenaro pepper plant propagation using seeds is restricted as it has a short span of viability with low germination rate and also it is highly susceptible to fungal and viral pathogens [3]. The natural vegetative propagation is limited in chilli, so conservation of genetic purity is very important through micropropagation. It has several medicinal properties like anti-inflammatory, analgestic, carminative, rubefacient and also possess powerful antioxidant, anti-mutagenic, anti-tumoural, hypoglycaemic, antifungal and antimicrobial activities have also been seen [4]. It is used as a counter irritant for the treatment of rheumatism, lumbago and neuralgia. It has been popularly used in both Ayurveda and Homeopathy system of medicine.

2. Materials and Method

Collection of Coir Pith

Coir pith required for the present study was collected from the coir mounds in the vicinity of a coir industry at Solavanthan, Madurai which is about 9 km away from Madurai Kamaraj University where coir processing is being carried out commercially.

Particle Size Distribution

As the above coir pith mass was formed of assorted particles of varied size it was sorted out into different grades based on the particle size. Coir pith mass was first sifted through a 2000 m sieve (ASTM 10). Bigger particles.

(>2000 m) which remained on the top of the sieve were separated. Rest of the coir pith was then sifted through a 1700 m sieve (ASTM 12). The particles (1700-2000 m) which stayed on the second sieve were then separated.

Porosity

A plastic cylindrical container of 250 ml capacity was taken. The bottom of the container was pierced with a fine needle so as to have ten holes uniformly distributed at the bottom of it. The holes were then closed with a water proof adhesive tape. Then the container was filled with the coir pith by gently tapping it till the coir pith fills the 250 ml mark. Then water was slowly dripped over the coir pith so that the coir pith was completely drenched and saturated (surface glistens). This process took several hours.

Water Absorption Capacity

Water absorption capacity of the coir pith refers to the quantity of water absorbed by a known volume of dry coir pith. It was assessed as per the procedure followed by Ross (2002) and Edsor (2005). Sun dried coir pith was used for this experiment.

Chemical Characteristics of Raw Coir Pith

Preparation of Aqueous Extract of Raw Coir Pith

Aqueous extract of raw coir pith was prepared as per the procedure followed by Ross (2002) and Edsor (2005) which is a modified procedure of Landis et al. (1989) and Lang (1996), The sieved pith was taken in a glass tray. It was wetted by spraying distilled water till one or two drops came out on squeezing it with hand. In a measuring cylinder distilled water was taken up to 333 ml mark. Then the wetted pith was introduced into the same cylinder till the total level reached 500 ml mark. The contents were continuously stirred with arod for 20 minutes. The mixture was then transferred to a glass tray and was hand squeezed. The resultant extract was collected and filtered through a Whatman No. 1 filter paper. The filtered extract was used for the analysis of pH, EC, sodium, potassium, calcium, chloride, phosphorus, iron, and phenol.

pH and Conductivity

pH and conductivity measurements were made on the aqueous extract of raw coir pith (vide Section 13 a) using a pH meter (Elico India 101 E) and conductivity meter (Elico India CM 180) respectively.

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Sodium, Potassium and Calcium

Sodium, potassium and calcium were analysed on the aqueous extract of raw coir pith (Section 13 a) using a flame photometer (Elico India, CL 22 D).

Chloride

Chloride content present in the aqueous extract of raw coir pith $_{was}$ estimated by Argentometric method (Section 4500-Cl^-B of Standard methods for the Examination of Water and Waste Water) (APHA / AWWA / WEF, 1998) and the values were expressed in mg Cl / L.

PHOSPHORUS

Phosphorus content of the aqueous extract of raw coir pith was determined by Stannous chloride method as described in Section 4500- PD of Standard Methods for the Examination of Water and Waste Water (APHA / AWWA / WEF, 1998).

Micropropagation of Capsicum SP

Sample Collection

The Capsicum fruits have been collected from the market, Madurai.

PROCESSING

The seeds were carefully collected and stored for further use.

Micropropagation

The in vitro seed germination was achieved on MS basal media. Previously the media was sterilized at 121ºC with 15 psi for 15 minutes. The cotton bed method was used with water and MS media for in vitro regeneration of plantlets from the collected seeds of Kandhari variety. All the materials used in culture work must be free from microbes. This is achieved by one of the following approaches flame sterilization, wiping with 70% ethanol and other surface sterilants. The Shoot tip and nodal region of in vitro derived plantlets were collected for the induction of multiple shooting. The surface sterilized seeds were transferred in Murashige and Skoog (MS) medium supplemented with 0.8% agar, 3% Sucrose and 1% myoinositol. The pH of the medium was adjusted to 5.7 and the maximum growth of plantlets was observed and it was sequentially subcultured in the media containing varying concentrations of BAP, IAA and NAA. Then subculturing process was carried out with the shoot tip and the nodal part of the plants. Then it was optimized with the maximum shootlets formed from the subcultured excised shoot tips.

3. Result and Discussion

Seeds which shown for in vitro regeneration were responded successfully. At the 15 th day seeds were germinated and by the 20 th day the maximum rate of growth was achieved. The plants which are grown in in vitro were utilized for In vitro cultivation studies. (Fari et al., 1990). The high frequency of germination was recorded on MS basal media, similar results was achieved in in vitro regeneration of chilli by (Zhao et al., 2003). In capsicum both apical and all nodal segment were used as primary explants, nodal explant is the most commonly used explant for producing micro shoots (Gunay et al., 1978). In present study high frequency of multiple shoot induction was achieved on auxillary bud explants. Media supplemented with 0.5mg/l BAP and 1.5mg/l of KIN shows high frequency.

Acclimatizing Period

Since coir pith is a very good transplanting medium, the hardening process was done with coir pith for the better proliferation of root and the water holding capacity of the coir pith enables the tissue cultured plants to grow well.

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