South African Journal of Botany 104 (2016) 1–5

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Efficacy of Zeatin, Kinetin and Thidiazuron in induction of adventitious root and shoot from petiole explants of sweetpotato cv. Brondal

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ARTICLE INFO

Article history:
Received 8 July 2015
Received in revised form 16 August 2015
Accepted 8 November 2015
Available online 17 December 2015

Edited by J Van Staden

Keywords:
Regeneration
Cytokinin
Zeatin
Petiole explant
Cultivar Brondal

ABSTRACT

In southern Africa, sweetpotato improvement through genetic engineering has been hampered by the lack of regeneration protocols of the most famous cultivars. The aim of this study was to develop an efficient and reproducible regeneration protocol of the sweetpotato cv. Brondal that is widely grown in Zimbabwe. In the regeneration study, the parameters for optimisation were petiole orientation (horizontal, vertical or inverted), Cytokinin type (Zeatin, Kinetin or Thidiazuron) and Cytokinin concentration (0.1 mg L^{-1}, 0.2 mg L^{-1}, or 0.4 mg L^{-1}), making a total of 27 treatments. In the first experiment, petioles were subjected to all 27 treatments to assess capacity to initiate root formation within three weeks. In the second experiment, only the effects of Cytokinin type and concentration were investigated to determine influence on root number, root length and shoot production. There was a significant ($p = 0.031$) interaction between orientation type and Cytokinin type, with the inverted orientation type producing the most number of roots (five roots/petiole) than the horizontal (three roots/petiole) or vertical (three roots/petiole) orientation types, regardless of the Cytokinin used. There was a significant ($p = 0.01$) interaction between Cytokinin type and Cytokinin concentration, with the Zeatin hormone consistently producing the most number of roots (five roots/petiole) than either Kinetin or Thidiazuron (average of three roots/petiole) regardless of the Cytokinin concentration used. Where root length was concerned, there was generally no significant difference between treatments (all treatments producing roots with lengths ranging from 0.1 to 7.0 mm) except for Kinetin 0.4 mg L^{-1} which consistently produced roots ranging 17–25 mm in length. For shoot production, all Zeatin had relatively higher (10.6%) shoot regeneration frequencies that either the Kinetin (3.3%) or Thidiazuron (0%). The greater the number of roots produced by a petiole, the greater the likelihood of shoot development on that petiole. In conclusion, the best treatments for root and shoot regeneration proved to be Murashige and Skoog media supplemented with Zeatin at 0.2 mg L^{-1} with petioles oriented in the inverted position.

1. Introduction

Sweetpotato (*Ipomoea batatas* L.) is a root crop that is well adapted to a wide range of agro-ecological conditions. It is high yielding, nutritious and has the ability to perform well under poor soil moisture and fertility (Gasura et al., 2010). In drought prone conditions common in southern Africa, these attributes are highly appealing, especially to resource poor farmers who often practise subsistence farming to sustain their families. Brondal cv. is one of the highest yielding sweetpotato cultivars in Zimbabwe with a yield potential of 40 tha^{-1} under irrigation. Brondal cv. produces purple skinned tubers with a white interior and a single tuber can weigh up to 2 kg. The deep purple skin colour of the cultivar is an indicative of high anthocyanin content and the presence of these anti-oxidants makes the consumption of this particular cultivar beneficial to human health (Rabah et al., 2004).

Plant tissue culture methods are widely used in the plant sciences and have several direct commercial applications. For instance, plant breeders have shortened breeding cycles by using anther culture to produce doubled haploid inbred lines and have used *in vitro* fertilisation methods to generate new varieties from crossing distantly related plants (Van den Bulk, 1991). Sweetpotato production in southern Africa has benefited immensely from tissue culture when the techniques have been applied to produce a large number of pathogen-free identical plants through the use of micro-propagation and meristem culture. Plant tissue culture also happens to be the regenerative power behind *Agrobacterium* mediated genetic transformation of plants. Genetic engineering helps to instil important traits such as disease and insect resistance, nutrient fortification, water-use efficiency and herbicide resistance into plants (Birch, 1997).

Sweetpotato cultivars in southern Africa are in dire need of both disease (mainly viral) and insect (mainly weevil) resistance as these can account for up to 60% (Karyeija et al., 2000) and 90% tuber yield losses (Mannion and Jansson, 1992). Breeding programs have been difficult

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http://dx.doi.org/10.1016/j.sajb.2015.11.001
0254-6299/

due to some genotypes which produce scanty flowers and in most cases do not flower at all (Mutasa et al., 2013). Gene constructs have been developed by several groups specifically to target weevil infestations (Rukarwa et al., 2014) and viral infections (Kashif et al., 2012) in the sweetpotato. However, these have largely not been implemented in the development of resistant varieties due to the absence of localised sweetpotato regeneration protocols which serve to underpin the success of any genetic transformation experiment, consequently limiting the improvement of the crop via genetic transformation means (Santa-Maria et al., 2009).

Sweetpotato is generally recalcitrant to regeneration efforts and regeneration is often genotype dependent (Yu et al., 2007; Manrique-Trujillo et al., 2013). For these reasons, it is often important to develop a regeneration protocol that can either be used for specific or general sweetpotato cultivars. In 1995, Gosukonda et al. (1995) attempted to develop a universal regeneration protocol that could be used to regenerate shoots from diverse sweetpotato genotypes using petioles as explants. The protocol was successfully extended to eight genotypes and one of the varieties regenerated had 78% shoot regeneration frequency. In the protocol Gosukonda et al. (1995), first induced root formation from the petiole before attaining shoot formation. It is with all this in mind that attempts we attempted to develop a regeneration protocol for sweetpotato cv. Brondal since no such protocol exists for the commercial varieties grown in southern Africa. The objective of this study was to develop a reproducible regeneration protocol for sweetpotato cv. Brondal using petioles as explants. In addition, the effect of petiole orientation, Cytokinin type (Zeatin, Kinetin and Thidiazuron) and concentration on root induction, root number, root length and ultimate shoot regeneration number in cv. Brondal were assessed.

2. Materials and methods

2.1. Plant material and their preparations for culturing

Field growing sweetpotato (cv. Brondal) vines were obtained from Agribiotech Pvt Ltd, Harare, Zimbabwe. All the leaves attached to the vines were removed and the leafless vines were cut into three nodal sections, 2–4 cm long. The nodal cuttings were then washed with soapy water to remove surface debris adhering to the explants. The plant segments were then placed under running tap water for 3 h in order to create an isotonic solution inside the segment. After the washing stage, the plant segments were surface sterilised in 20% jik for 15 min. This procedure was performed in the laminar airflow hood. The sterilised vine segments were then rinsed thrice with sterile distilled water to remove the disinfecting jik. Sterile forceps were used to move the sterilised stem sections into sterile jam jars containing a basal media of cotton-wool with 2% sucrose. The cultures were then taken to the growth room. After four weeks, the cultured vines produced sufficient mother stocks to be sub-cultured onto plain solid (Murashige and Skoog, 1962) media with 2% sucrose. The plants were then continuously subcultured every 4–6 weeks. Before the start of each experiment, the petiole explants isolated from mother stock plants were pre-conditioned by culturing them onto 0.2 mg L^{-1} 2.4 D for three days. After that period, only those petiole pieces that would have developed a swollen bump at one end of the explant were advanced to the experimental stages. After the petioles had been cultured onto the respective media treatments, the petiole cultures were then moved to the growth room where temperature was kept at 25 ± 3 °C and a 16 hour light, 8 hour dark, photoperiod was maintained.

2.1.1. Effect of petiole orientation on root initiation

Pre-conditioned petioles with a swollen tip were laid out into a completely randomised design with three factors of three levels. Petioles were placed either horizontally, vertically or inverted as described by Gosukonda et al. (1995) onto MS media with either Zeatin (0.1 mg L^{-1}, 0.2 mg L^{-1}, 0.4 mg L^{-1}), Kinetin (at 0.1 mg L^{-1}, 0.2 mg L^{-1}, 0.4 mg L^{-1}), or Thidiazuron (0.1 mg L^{-1}, 0.2 mg L^{-1}, 0.4 mg L^{-1}) making a total of 27 treatments. Orientation was in respect of the swollen tip on the petiole. After 3 weeks of culture, the number of roots that developed on each petiole was recorded.

2.1.2. Effect of Zeatin, Kinetin and Thidiazuron on shoot induction from petiole explants

Pre-conditioned petioles with a swollen tip were placed in an inverted position onto MS media with either Zeatin (0.1 mg L^{-1}, 0.2 mg L^{-1}, or 0.4 mg L^{-1}), Kinetin (0.1 mg L^{-1}, 0.2 mg L^{-1}, or 0.4 mg L^{-1}), and Thidiazuron (0.1 mg L^{-1}, 0.2 mg L^{-1}, or 0.4 mg L^{-1}) making a total of 9 treatments. The experiment was laid out in a randomised complete block design with three blocks. Root number and root length/expansion was assessed 4 weeks after culture. After 8 weeks, the number of shoots that developed in each treatment was also recorded.

2.2. Statistical analysis

Square root transformation was done to improve the normality of data. Data was then subjected to analysis of variance using Genstat software. Means were differentiated by using 5% least significant difference. Shoot regeneration frequency was computed as the proportion of explants showing shoots multiplied by 100. Both experiments were repeated three times to determine reproducibility of the results.

3. Results

3.1. Effect of petiole orientation and hormone type and concentration on sweetpotato petiole root initiation

There was a significant ($p = 0.031$) interaction between petiole orientation and type of Cytokinin used on root initiation from petioles explants. Across all three Cytokinin types used, the inverted orientation produced the highest number of root initiation (five roots/petiole) when compared to either the horizontal or vertical orientations which produced an average of three roots/petiole each (Fig. A.1). Although the vertical orientation type tended to produce more roots than the horizontal orientation type in all cases except when used in combination with the Zeatin hormone, statistically, there was no significant difference in root initiation between the vertical and horizontal treatments regardless of the Cytokinin type used. Inverted petioles with Zeatin at any concentration inevitably produced the greatest number of root initials compared to any other treatment combination.

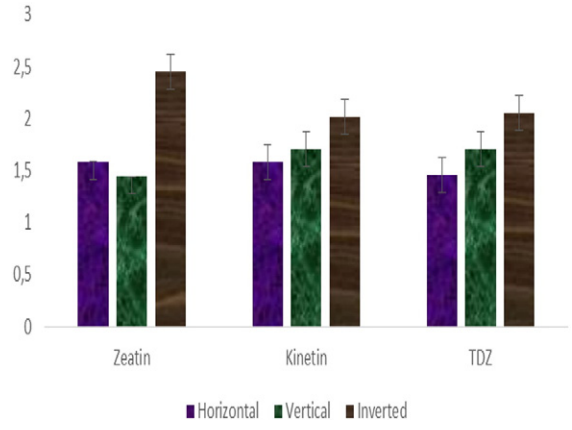

Fig. A.1. Effect of petiole orientation across three Cytokinin types on root initiation in sweetpotato cv. Brondal 21 days after culture.