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Synthetic seeds for in vitro preservation of *Asparagus officinalis* L.

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Abstract

Background Asparagus (*Asparagus officinalis*) is a perennial vegetable of economic importance for its high nutritional and medicinal value. Male plants are more desirable because of their higher spear yield. Therefore, the aim of this study was to evaluate the efficiency of the gibberellin inhibitors; paclobutrazol (PBZ) and cycocel (CCC) for in vitro preservation by encapsulation of in vitro-derived shoot tips of the superior germplasm of the first generation of asparagus male hybrid; cultivar Mary Washington 500W.

Methods An efficient technique for synthetic seeds production was achieved, consisting of 3% (w/v) Na-alginate dissolved in water, Murashige and Skoog (MS) medium, MS medium with the growth inhibitors; PBZ and CCC at different concentrations, and 0.1 M calcium chloride ($CaCl_2.2H_2O$). Synthetic seeds were stored at 4 ℃ and then cultured after different storage durations (0, 2, 4, 8, 12, 16, 20 and 24 weeks) on MS medium supplemented with 1 mg l^{-1} kinetin, 0.2 mg l^{-1} naphthalene acetic acid and 0.5 mg l^{-1} gibberellic acid for shoot tip multiplication; then, recovered shoots were transferred to MS medium supplemented with 1 mg l^{-1} indole butyric acid and 0.5 mg l^{-1} PBZ for rooting.

Results The beads containing 1 mg l^{-1} PBZ considered the optimum for producing complete well-developed plantlets of *Asparagus officinalis* from recovered shoot tips after 24 weeks of storage that successfully acclimatized in the greenhouse.

Conclusions This protocol is efficient for in vitro preservation by encapsulation of shoot tips and regeneration of *Asparagus officinalis* F1 male hybrid (Mary Washington 500w), using anti-gibberellin plant growth regulators within the beads to prolong the duration of storage and provide a continuous supply of the plant.

Keywords Asparagus, Artificial seeds, Encapsulation, Growth inhibitor, Paclobutrazol, Cycocel

Background

Synthetic seeds are artificially encapsulated explants of any active meristematic tissues that can convert into a whole plant, either in vitro or ex vitro, and retain this ability after preservation (Nongdam 2016). Synthetic seeds technology is promising for plant preservation and multiplication, particularly for no-seed producing plants, plants do not produce viable seeds or require vegetative propagation for maintaining desirable and superior characteristics. This technology offers many advantages such as reducing cost and space, easy to handle, germplasm conservation of rare and economically important plants, continuous and seasonal independent multiplication of genetically stable elite plant species, and the exchange of germplasm between countries (Abbas and Alhasan 2022). It has been applied successfully on a variety of plants and commercially important horticultural crops, including vegetable crops (Sahoo et al. 2012; Abbas and Alhasan 2022).

Genus *Asparagus* is a perennial vegetable from family Asparagaceae (www.ipni.org). Asparagus *officinalis* L., known as "garden asparagus", is economically

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the most important species of *Asparagus*, which is an expensive and highly nutritionally valuable vegetable. The traditional propagation of *Asparagus* is inefficient; seed germination rate is low and produces equal numbers of male and female plants and vegetative propagation by mechanical division of the rhizome is time and money-consuming and produces a very limited number of clonal copies of the plant. Also, this method causes a risk of spreading diseases (such as *Fusarium* sp.). *Asparagus officinalis* is a dioecious plant; therefore, it is impossible to use sexual reproduction by seeds for the propagation of new elite genotypes of asparagus (Encina and Regalado 2022). Asparagus male plants have more commercial advantages over the female plants, they give higher yields, are longer lived and are less susceptible to disease, while female plants put much energy into producing seeds (Lo´pez-Anido and Cointry 2008).

In vitro preservation of asparagus material under controlled conditions is the best solution for producing the desirable male plants; it is safe and avoids the biotic and abiotic risks of the in situ or ex situ conservation of asparagus in field plantations. Moreover, seed banks are difficult to maintain due to the low viability of seeds (Encina and Regalado 2022). Recently, Abbasi et al. (2020) developed synthetic seeds from in vitro grown apical buds and somatic embryos of *Asparagus officinalis* and they were stored under in vitro conditions up to 90 days, then successfully germinated and converted into seedlings.

Among preservation strategies used to delay the growth and development of in vitro cultures is the use of anti-gibberellin growth inhibitors [e.g. paclobutrazol (PBZ) and cycocel (CCC)] (Huang et al. 2014; Hajihashemi and Geuns 2017; Ramírez-Mosqueda et al. 2019). Anti-gibberellin growth regulators inhibit gibberellin biosynthesis at different stages of the biosynthetic pathway, leading to decreasing the growth and slowing down the rate of apical development by reducing the amount of active gibberellins (PirastehAnosheh et al. 2016). PBZ belongs to the triazole chemical group and is known as anti-gibberellin. The application of PBZ blocks the synthesis of gibberellins, transports in the xylem and can be absorbed by the stem, leaves or roots (Seesangboon et al. 2018). Also, CCC acts antagonistically to gibberellic acid and inhibits stem elongation (Amoanimaa-Dede et al. 2022).

The objective of this work was the in vitro preservation of "super-male" genotypes from an elite *Asparagus officinalis* F1 male hybrid (Mary Washington 500w), by synthetic seed technology using growth inhibitors (PBZ and CCC) for the first time to prolong the duration of storage and provide a continuous supply of the plant.

Methods

Plant material

Seeds of F1 male hybrid of *Asparagus officinalis* (Mary Washington 500 W') were sown in an artificial medium of peatmoss, vermiculite and sand (1: 1: 1 v/v/v) in plastic pots in the greenhouse as described by Sallam (2019). After nine months of seeds germination, nodal segments of young spears from F1 male hybrid plants were surface sterilized and used as explants for the micropropagation of the plant.

Synthetic seeds production

Shoot tips were separated from in vitro cultures and suspended in a sterile matrix of Murashige and Skoog (1962) medium (MS medium; Caison, USA), supplemented with 100 mg l^{-1} myo-inositol (Fluka AG, Switzerland) and 30 gl^{-1} sucrose in 3% (w/v) sodium alginate (Na-alginate; CDH, India), and fortified by different treatments of growth inhibitors (PBZ and CCCat 0.5, 1 and 2 mg l^{-1}; min. 90%, Sigma-Aldrich, Germany). After that, they were transferred by dropping into a sterile 100 µM calcium chloride ($CaCl_2.2H_2O$; Alpha Chemika, India) solution with the help of a sterilized 1000 µl micropipette (Boeco, Germany) to accomplish the encapsulation of shoot tips. The beads were shaken for 30 min for proper beads formation, then washed with sterile distilled water to remove the excess solution of $CaCl_2.2H_2O$ and placed on a sterile filter paper for removing excess water.

Synthetic seeds storage and germination

Synthetic seeds were incubated at a temperature of 4 ± 1 °C for 24 weeks. For germination, the synthetic seeds were cultured after each storage duration (0, 2, 4, 8, 12, 16, 20 and 24 weeks) on MS medium supplemented with 100 mg l^{-1} myo-inositol, 30 g l^{-1} sucrose, 200 mg l^{-1} glutamine, 1 mg l^{-1} kinetin (KIN), 0.2 mg l^{-1} naphthalene acetic acid (NAA) and 0.5 mg l^{-1} gibberellic acid (GA_3) for shoot tip germination and multiplication; then, recovered shoots were transferred to MS medium supplemented with 1 mg l^{-1} indole butyric acid (IBA) and 0.5 mg l^{-1} PBZ for rooting (Sallam 2019). Plant growth regulators were purchased from Sigma-Aldrich, Germany (min. 90%). The pH of the medium was adjusted to 5.7 ± 1 before adding phytagel (2.5 g/l; Duchefa, Haarlem, the Netherlands) and dispensed into jars, then sterilized under a pressure of 1.1 kg/cm^3 and a temperature of 121 °C for 15 min (Wisd. Laboratory Instruments autoclave).

Cultures were maintained in the growth chamber at 25 ± 2 °C under light conditions of 16-h photoperiod provided by cool-white, fluorescent lamps (2500–3000 Lux). Non-encapsulated shoot tips and encapsulated shoot tips

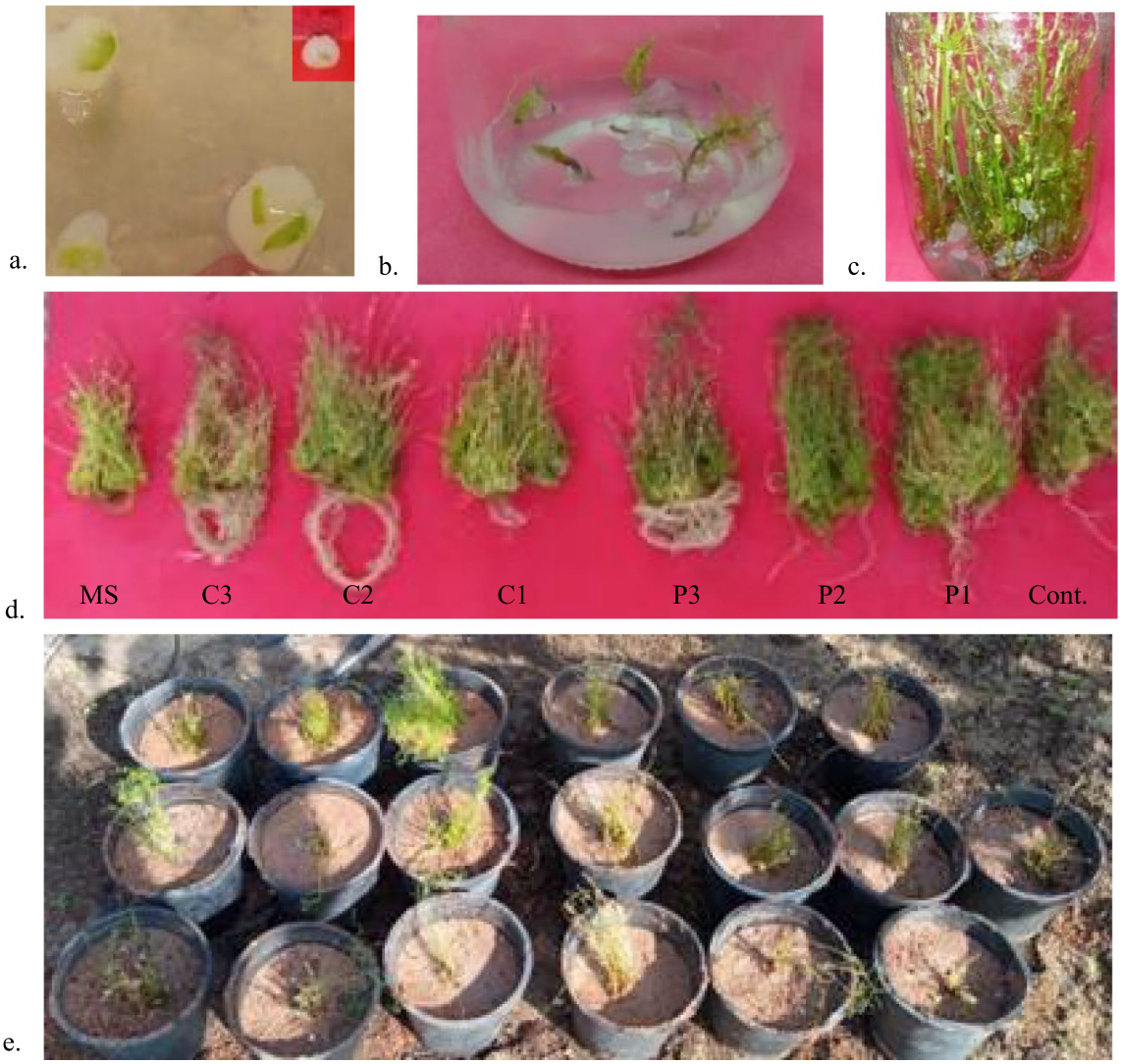

Fig. 1 In vitro preservation of *Asparagus officinalis*. **a.** Synthetic seeds, **b.** germinated synthetic seeds, **c.** multiplication of shoots, **d.** rooting of shoots on different treatments; MS: Control without growth inhibitors, C1: 0.5 mg l^{-1} CCC, C2: 1 mg l^{-1} CCC, C3: 2 mg l^{-1} CCC, P1: 0.5 mg l^{-1} PBZ, P2: 1 mg l^{-1} PBZ, P3: 2 mg l^{-1} PBZ, Cont.: control (unencapsulated shoot tips) **e.** acclimatized plantlets in the greenhouse

without growth inhibitors were served as control. The duration of germination (days), germination percentage (%), shoots number per explant, shoot and root length (cm), percentage (%) of rooting and acclimatization were evaluated after 0, 2, 4, 8, 12, 16, 20 and 24 weeks of storage and data were recorded after eight weeks from culturing. The complete plantlets were slowly transferred to *ex vitro* conditions in the greenhouse into pots containing peatmoss and sand (1: 1 v/v), covered with plastic covers and watered regularly twice a week, then holes were made in the covers gradually until they were completely removed after a month.

Experimental design and statistical analysis

In this study, experiments were carried out twice based on a completely randomized experimental design with at least six replicates per treatment, and each replicate comprised of five explants. Data were analysed using the analysis of variance (ANOVA) with SPSS software (SPSS Inc. Version 20). Mean values were compared using Duncan's multiple range test at a probability level of 0.05.

Results

For producing synthetic seeds of *Asparagus officinalis* "super-male" hybrid (Mary Washington 500w), shoot tips produced from in vitro cultures were used as explants (Fig. 1a). After each storage duration, the synthetic seeds

Table 1 Effect of synthetic seed matrix supplemented with MS medium and PBZ or CCC at different concentrations on the duration of germination of encapsulated shoot tips of *Asparagus officinalis* after different storage durations

Matrix composition	Storage duration (week)							
	0	2	4	8	12	16	20	24
	Duration of germination (days)							
Non-encapsulated shoot tips	3.00e							
MS medium	3.33e	3.33d	3.67d	3.00c	3.00f	3.00e	4.00e	5.00c
MS medium + 0.5 mg l^{-1} PBZ	9.33d	13.00c	14.67c	15.00b	15.00e	15.00d	32.33bc	35.00b
MS medium + 1 mg l^{-1} PBZ	12.67cd	15.00bc	15.00c	15.00b	19.00de	21.00c	21.00d	35.00b
MS medium + 2 mg l^{-1}PBZ	14.00bc	17.00bc	19.00bc	19.00b	25.33bc	31.67b	31.67c	36.67b
MS medium + 0.5 mg l^{-1} CCC	9.33d	15.00bc	17.00c	19.00b	23.67cd	27.00b	27.00cd	36.67b
MS medium + 1 mg l^{-1}CCC	17.33b	18.33b	22.33ab	28.33a	30.00ab	36.67a	38.33ab	40.00ab
MS medium + 2 mg l^{-1} CCC	23.67a	25.33a	27.00a	31.67a	35.00a	38.33a	43.33a	45.00a

of *Asparagus officinalis* were cultured on MS medium supplemented with 100 mg l^{-1} myo-inositol, 30 g l^{-1} sucrose, 200 mg l^{-1} glutamine, 1 mg l^{-1} KIN, 0.2 mg l^{-1} NAA and 0.5 mg l^{-1} GA_3 for shoot tip germination and multiplication. The recovered shoots were transferred to MS medium supplemented with 1 mg l^{-1} IBA and 0.5 mg l^{-1} PBZ for rooting. Data were recorded after eight weeks from culturing. Table 1 shows the effect of synthetic seed matrix supplemented with PBZ or CCC at different concentrations on the duration of germination of encapsulated shoot tips of *Asparagus officinalis* after different storage durations. Comparing non-encapsulated shoot tips with alginate-encapsulated ones (synthetic seeds), either with or without growth inhibitors, the encapsulation exhibited delayed germination (fresh shoot emergence). However, the presence of PBZ or CCC in the bead significantly increased the duration of germination, in comparison with that without growth inhibitors. Increasing the concentration of PBZ or CCC significantly delayed the germination of synthetic seeds. Storage duration of synthetic seeds reached 24 weeks. The maximum duration of germination of synthetic seeds was observed using 2 mg l^{-1} CCC in the beads, including all the studied storage durations reaching a mean of 45 days after 24 weeks of storage.

Table 2 represents the effect of synthetic seed matrix supplemented with PBZ or CCC at different concentrations on the germination percentage after different storage durations. Hundred per cent of non-encapsulated shoot tips germinated, while the percentage of germination decreased gradually with increasing the duration of storage for the encapsulated shoot tips in all tested matrix compositions. However, the highest germination percentage was observed in media without growth inhibitors for all durations. This was accomplished by the rapid germination. Synthetic seeds with CCC ranked next, during all the storage durations, followed by that containing PBZ. They gave 100% germination at 0 and 2 weeks of storage and then decreased gradually by time. The concentration of 0.5 mg l^{-1} CCC was superior, it

Table 2 Effect of synthetic seed matrix supplemented with MS medium and PBZ or CCC at different concentrations on the germination percentage of encapsulated shoot tips of *Asparagus officinalis* after different storage durations

Matrix composition	Storage duration (week)							
	0	2	4	8	12	16	20	24
	Germination percentage (%)							
Non-encapsulated shoot tips	100							
MS medium	100	100.0a	81.0a	81.00a	80.0a	61.0a	12.5a	5.0d
MS medium + 0.5 mg l^{-1} PBZ	100	88.6b	50.0d	50.00c	27.7d	25.0d	10.0d	0.0f
MS medium + 1 mg l^{-1} PBZ	100	78.0c	45.0e	45.00c	25.0e	25.0d	8.0d	2.0e
MS medium + 2 mg l^{-1} PBZ	100	73.0d	30.0f	30.00d	24.6e	23.5d	5.0d	2.0e
MS medium + 0.5 mg l^{-1} CCC	100	100.0a	77.0b	77.00a	55.5b	45.4b	22.0a	20.0a
MS medium + mg l^{-1} CCC 1	100	100.0a	75.0b	72.33ab	46.0c	44.4b	22.0a	16.0b
MS medium + mg l^{-1} CCC 2	100	100.0a	66.5c	66.50b	45.0c	36.0c	16.0b	12.5c

Table 3 Effect of synthetic seed matrix supplemented with MS medium and PBZ or CCC at different concentrations on shoots number and length, root length, percentage of rooting and acclimatization of encapsulated shoot tips of *Asparagus officinalis* after eight weeks from culturing

Matrix composition	Shoots number/explant	Shoot length (cm)	White functional roots (%)	Root length (cm)	Acclimatization (%)
Non-encapsulated shoot tips	7.38b	7.89b	79.17b	19.60a	10.0de
MS medium	3.25e	3.78e	0.00e	0.00c	0.0e
MS medium + 0.5 mg l^{-1} PBZ	6.88b	6.72c	63.75c	4.25bc	27.5d
MS medium + 1 mg l^{-1} PBZ	5.13c	6.17c	90.00ab	5.50bc	50.0bc
MS medium + 2 mg l^{-1}PBZ	3.88de	3.83e	95.00a	7.00bc	77.5ab
MS medium + 0.5 mg l-1 CCC	9.50a	9.28a	0.00c	0.00c	0.0e
MS medium + 1 mg l^{-1} CCC	6.75b	7.89b	22.5d	10.00b	37.5cd
MS medium + 2 mg l^{-1} CCC	5.00cd	5.17d	20.00d	4.75bc	60.0b

retained this capability until 24 weeks of storage and gave the highest germination percentage (20%), compared to the control and other treatments (Fig. 1b).

Concerning the vegetative growth of shoots and roots, data in Table 3 show that the highest shoot multiplication and elongation were observed in the shoots derived from beads containing CCC at 0.5 mg l^{-1} (9.5 shoots of 9.28 cm length), and shoots number and length decreased with increasing the concentration of CCC; however, these treatments gave the lowest percentage of white functional roots (0–22.5%), and the shoots were weak and light green in colour with small leaves. The treatment of 0.5 mg l^{-1} PBZ ranked next concerning the number and length of shoots; however, the shoots were healthy, thick and dark green in colour with broad leaves (Fig. 1c) and high percentage of functional white roots (63.75%). However, the acclimatization percentage was low with this treatment, and it recorded only 27.5%.

Although the treatment of 1 mg l^{-1} PBZ gave lower number and length of shoots (5.13 shoots of 6.17 cm / explant), it gave high rooting percentage of strong well-developed white functional roots (90%) and 50% of plantlets successfully acclimatized in the greenhouse. The highest concentration of PBZ (2 mg 1^{-l}) recorded the highest percentage of white functional roots (95%) and acclimatization percentage reached 77.5%, but the shoots were weak and short and pale green in colour. Therefore, the beads containing 1 mg l^{-1} PBZ considered the optimum for producing complete well-developed plantlets of *Asparagus officinalis* (Fig. 1d and e).

The control treatment of MS medium without growth inhibitors induced weak pale green shoots with callus on the base of shoots and failed to induce rooting. Also, vitrification was seen on the shoots with the control treatment, while no vitrification was observed with the application of growth inhibitors in the beads.

Discussion

Synthetic seed technology provides high quantity and low-cost short- to mid-term storage of elite germplasms (Kikowska and Thiem 2011). Rationale of choosing the shoot tip as explant for encapsulation of *Asparagus officinalis* was mainly due to its active meristematic tissues that have the ability to initiate cultures amongst different explants. It also provides earlier and rapid regeneration, as seen in several studied plants (Gantait et al. 2015). In addition, using MS medium in the synthetic seed matrix as a basal medium is due to its positive effect in enhancing germination, causing effective conversion of the encapsulated explants into complete plantlets, as reported by Gantait et al. (2022). After each storage duration, the synthetic seeds were cultured on MS medium supplemented with 100 mg l^{-1} myo-inositol, 30 g l^{-1} sucrose, 200 mg l^{-1} glutamine, 1 mg l^{-1} KIN, 0.2 mg l^{-1} NAA and 0.5 mg l^{-1} GA_3 for shoot tip germination and multiplication. The recovered shoots were transferred to MS medium supplemented with 1 mg l^{-1} IBA and 0.5 mg l^{-1} PBZ for rooting. In a previous study of Abbasi et al. (2020), they developed apical buds from *Asparagus officinalis* in vitro culture on MS medium supplemented with also 1 mg l^{-1} KIN but in combination with 1 mg l^{-1} 2,4-D, which had the potential to create synthetic seeds. They found that seeds grown in MS medium germinate and convert to seedlings at a faster rate than seeds grown in ½ MS medium. The highest germination percentage and rapid germination were observed without growth inhibitors for all durations. This observation is in harmony with Soni and Sharma (2017), who studied the encapsulation of *Asparagus racemosus*, that showed 94.9% germination of beads within three weeks and germinated seedlings formed complete plantlets with developed root–shoot system.

Synthetic seeds with CCC ranked next, followed by that containing PBZ, for all storage durations and the

concentration of 0.5 mg l^{-1} CCC was superior. Nower et al. (2007) studied the effect of growth retardants, using abscisic acid (ABA), PBZ and CCC on the growth ability of encapsulated buds of *Pyrus communis*, the maximum response was obtained on MS medium supplemented with 0.5 mg l^{-1} CCC and control (without growth retardants) after 16 weeks of storage. They reported that the preservation method by growth retardants slows down cell metabolism and prevents somaclonal variation. Pourmohammad et al. (2013) reported that the application of CCC enhanced seed germination and growth due to the increase in respiration potential, ATP generation and protein synthesis (PirastehAnosheh et al. 2016). The effectiveness of CCC depends upon several factors, such as concentration, time of application, environmental conditions, and nutritional status of the plant species (PirastehAnosheh et al. 2016).

Regarding the effect of PBZ on increasing the duration of germination and decreasing the percentage of germination, this could be explained by the role of PBZ in blocking the conversion of ent-kaurene to ent-kaurenoic acid during the gibberellin biosynthesis pathway by inhibiting kaurene oxidase (Detpitthayanan et al. 2019). This causes delaying of germination and inhibits germination and plant growth (Bisht et al 2018; Mendes et al. 2021). In vitro preservation of many plants such as citrus would be more efficient with the application of PBZ as a growth inhibitor, which reduces contamination and increases the period of preservation, thus reducing the labourers and materials cost due to less manipulation (Mendes et al. 2021).

The highest shoot multiplication and elongation were observed in the shoots derived from beads containing the lowest concentration of CCC (0.5 mg l^{-1}), which decreased with increasing the concentration, and the shoots were weak and light green in colour with small leaves and the lowest percentage of white functional roots. The inhibiting effect of high concentrations of CCC on shooting, which is considered as the most important morphological changes of CCC application, gave evidence on the anti-gibberellin mode of action of CCC. It is gibberellin biosynthesis inhibitor that blocks ent-kaurene synthesis from geranylgeranyl diphosphate in gibberellin biosynthesis pathway. It inhibits mainly the activity of copalyl diphosphate synthase enzyme and ent-kaurene synthase enzyme but in a smaller degree (PirastehAnosheh et al. 2016). The treatment of 0.5 mg l^{-1} PBZ ranked next; however, the shoots were healthy, thick and dark green in colour with broad leaves and high percentage of functional white roots. This result is in harmony with Indrayanti et al. (2018), who reported that PBZ improved number of shoots and leaf shape, plant height of banana after six months of in vitro storage. Moreover, Abdalla et al. (2021) reported that PBZ reduces plant growth without being toxic. It may also induce changes in the morphology of leaves inducing thicker and larger leaves, smaller stomatal pores and increased rooting density, that may increase the plant environmental stress tolerance and disease resistance.

It was observed that adding PBZ to the beads stimulated the growth of roots in the multiplication medium and its highest concentration negatively affected the vegetative growth of shoots. Similarly, plantlet regeneration was promoted with improved performance by PBZ in in vitro cultures of *Pinus massoniana* after long-term preservation (Wang and Yao 2020). PBZ is known to promote the thickening of roots and reduce length of shoots and roots as found for *Zygopetalum crinitum* orchid (Gimenes et al. 2018). Optimum rooting of in vitro shoot cultures of *Pinus massoniana* and Greenberry (*Rubus brasiliensis*) was performed by adding PBZ to an exogenous auxin with increased rooting rate and number as reported by Wang and Yao (2021) and Bueno et al. (2021), respectively. Furthermore, PBZ has a positive impact on root growth of *Dendrobium nobile* orchid and therefore improves the transfer of plantlets from in vitro culture to pots (Wen et al. 2013). It is well known that PBZ inhibits the biosynthesis of gibberellins, hence reducing cell elongation (Fletcher et al. 2010; Hajihashemi and Geuns 2017). However, the reduction in endogenous gibberellins by PBZ has been reported to promote shoot proliferation in vitro (Chen and Chang 2003). The optimum application rate of PBZ may depend on many factors including species, method of cultivation, growth stage and method of application. In general, PBZ acts as a stress protectant by stabilizing membrane and relative water content, maintaining photosynthetic activity by enhancing the level of antioxidant activity, osmolytes and endogenous hormones, and thus enhancing the growth and yield (Abdalla et al. 2021).

The positive effect of CCC on the growth could be due to various reasons such as stomatal closure, increased chlorophyll content and intercellular CO_2 concentration, and stimulatory changes in other physiological and biochemical attributes (PirastehAnosheh et al. 2012). CCC can also stimulate root growth, reduce transpiration, increase water use efficiency, and prevent chlorophyll destruction (Rajala 2003; Wang et al. 2010). Increased levels of protein, proline, sugar and antioxidant enzyme activities in plants under stress conditions are natural responses, which can help plants better tolerate the stress. Furthermore, the enhanced antioxidant enzyme activities in response to cycocel application may also protect their photosynthetic machineries

against damages caused by ROS during water-deficit conditions (Ashraf 2010; Wang et al. 2010; PirastehAnosheh et al. 2012).

The vitrification that was observed in the shoots of *Asparagus officinalis* with the control treatment and disappeared with the application of growth inhibitors in the beads is confirmed by Sallam (2019), who found that vitrification was eliminated in the shoots of *Asparagus officinalis* when PBZ was used at 1 mg l^{-1}, this treatment also produced a high percentage of white-rooted plants (79.50%) in the presence of IBA at 0.5 mg l^{-1} to IBA in the medium.

Conclusions

The present study supports the efficiency of the proposed protocol for in vitro preservation by encapsulation of shoot tips and regeneration of *Asparagus officinalis* F1 male hybrid (Mary Washington 500w) plantlets using anti-gibberellin plant growth regulators within the beads. The treatment of 1 mg l^{-1} PBZ gave 90% rooting of strong well-developed white functional roots, and the plantlets were successfully acclimatized in the greenhouse. This protocol when applied may prolong the duration of storage and provide a continuous supply of elite plants.

Abbreviations

ANOVA	Analysis of variance
$CaCl_2.2H_2O$	Calcium chloride
CCC	Cycocel
GA_3	Gibberellic acid
IBA	Indole butyric acid
kin	Kinetin
MS	Murashige and Skoog
NAA	Naphthalene acetic acid
PBZ	Paclobutrazol

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Author contributions

ARS shared in the research proposal and did the laboratory work. GAH shared in the research proposal, analysed the data, wrote, and revised the manuscript. SAB shared in the research proposal. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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