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FACTORS AFFECTING ASPARAGUS (*Asparagus officinalis* L.) ROOT DEVELOPMENT *IN VITRO*

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Abstract. Parental lines are propagated *in vitro* in asparagus breeding. Additionally, in order to obtain super males which are needed for breeding all-male hybrids, anther culture is applied. Healthy and viable asparagus parental plants developed from *in vitro* culture should contain well formed storage roots. There are many factors affecting asparagus rooting. Only with the healthy explant materials, proper culture medium and growth conditions can asparagus plantlets be produced and later successfully grown in the greenhouse. In this article, obstacles and factors affecting asparagus rooting *in vitro* as well as trends in developing rooting technique are summarized.

Key words: asparagus, rooting *in vitro,* growth regulators, media

BACKGROUND

Asparagus (*Asparagus officinalis* L.) is a popular vegetable because it is one of the first field crops to be harvested in spring and it can provide both growers and consumers with an early-season fresh commodity. As a dioecious crop, asparagus is inevitably cross-pollinating. Male and female flowers are borne on different plants. Generally, male plants have more commercial advantages over the female plants. They have higher productivity [Falloon and Nikoloff 1986] and produce more stalks [Gonzalez Castanon 1990]. Female plants live shorter, emerge later in spring and produce fruits. These fruits compete with the crowns and roots for nutrients. All-male hybrids produce higher yield, have better disease tolerance, greater longevity and definitely no seed is produced [Falloon 1982]. In order to obtain all-male hybrids, the production of supermale is the key. Nevertheless, supermale rarely exists in nature. Therefore, supermale plants are produced through pollen or anther culture [Doré 1977]. In asparagus *in vitro* culture, the development of storage roots is essential. Plantlets without well developed storage roots

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might fail to survive after transplantation. Hence well-formed and healthy storage roots are the precondition for plantlet survival and growth in the future.

BARRIERS OF ASPARAGUS ROOT DEVELOPMENT *IN VITRO*

The obstacles of rooting *in vitro* can be categorized into three aspects:

1. low rooting rate
2. big amount of abnormal roots
3. low survival rate after transplanting to the greenhouse

Rooting rate mainly depends among others on: cultivars and genetic variation among individuals within a cultivar [Tsay et al. 1980; Shi et al. 1995; Gonzalez Castanon 2002], type of explants [Shen et al. 1995], the history of pre-culture before subcultivating plantlets on the last rooting medium [Yakuwa et al. 1982], rooting medium with different growth regulators [Inagaki et al. 1981; Lin et al. 2008; Saharan 2010] as well as subculture conditions [Inagaki et al. 1983; Mamiya and Sakamoto 2000]. On account of the interaction among these factors, the rooting rate of plantlets *in vitro* is always fluctuating, which may give rise to a low survival rate when plantlets are transplanted to the greenhouse. Therefore, improving the rooting technique *in vitro* has inevitably become an important pathway to increase the plantlet survival rate in the greenhouse.

FACTORS AFFECTING ASPARAGUS ROOT DEVELOPMENT *IN VITRO*

Genotype and cultivar

Among different factors affecting rooting culture *in vitro*, genotype and cultivar are of prime importance.

Firstly, genotype has strong impact on callus formation from anther culture [Chen et al. 1997]. Different cultivars also gave various callus induction rates. Lin et al. [2007] found that callus inductivity through anther culture varied from different cultivars and individual plants in one cultivar. 'UC 155' obtained higher callus inductivity – 18.9% in comparison to 'UC 142', 'Gijnlim', 'Thielim' – 11.6%, 10.3%, 5.8%, respectively. The highest callus inductivity of individual plants of 'UC 155' amounted to 83.5%. In the study of Tang et al. [2011], 'Thielim' gave the highest callus inductivities (46%) while 'Backlim' had the shortest callus induction time (33 days) among five cultivars.

Secondly, genotype gives strong influence on their subsequent growth on the rooting medium and even their adaptation to the greenhouse conditions. In the study of Krzyżanowska et al. [1995], four breeding lines were used to study the effects of genotypes on plantlet regeneration and rooting abilities. All lines were found to be intensively proliferated, with line 91/89M the most intensive and 1/89F the weakest. 91/89M obtained the highest rooting rate (85.7% – 100%) but 1/89F the lowest. Fortes et al. [1997] and Gebler [2005] investigated the influence of genotypes on *in vitro* rooting of asparagus. Fortes et al. [1997] found that clone 'M14' had better rooting ability than the hybrid 'Deco' (M14 X G27). Gebler [2005] illustrated that only five out of eleven plant-

lets derived from different single embryos had rooting abilities. Pontaroli and Camadro [2005] studied the callus growth and plant regeneration from two selected clones of asparagus: 'Argenteuil 265' and 'Argenteuil 357'. From this experiment, 100% rooting rate from clone 'Argenteuil 265' was obtained.

Explants

Different parts of mother plants can be chosen to establish an asparagus clone through *in vitro* culture, e.g. anthers [Doré 1977; Tsay et al. 1980; Inagaki et al. 1981; Reuther 1983], shoot segments [Harada and Yakuwa 1983], bud clusters [Kohmura et al. 1994]. Callus induction efficiency varies in explant materials.

Recent studies on asparagus *in vitro* culture conducted in China proved the importance of explant material selection. Shoot basal segments were proved to be better explants compared to shoot middle or apical segments after some studies by Cai et al. [2005], Lin et al. [2006a], Chen [2007]. Nevertheless, Lin et al. [2008] clarified shoot tips could be ideal explant materials.

Explants derived from different parts of the mother plants, after initiate growth on the callus induction medium, can be developed into plantlets. However, not all types of explants are favorable for root stimulation. Shen et al. [1995] found that when both clustered shoots (with 2–5 shoots) and a single shoot tip were used as explants to be subcultured on 1/2 MS medium with 0.1 $ml \cdot L^{-1}$ NAA, a higher rooting percentage (78.9%) was received from clustered shoots in comparison to that from a single shoot tip, which only gave 25.7% rooting. Fortes et al. [1997] and Gebler [2005] illustrated similar results. Fortes et al. [1997] studied the influence of young shoot number on asparagus rooting. It was reported that four-shoot explants rooted better than those with one, two or three shoots. Longer roots were also observed from the explant with four shoots. Gebler [2005] stated that none of the single shoot explant could develop storage roots.

Conner et al. [1990] described that an ideal type for micropropagated asparagus plantlets should consist of an *in vitro* minicrown with 4–6 shoots 1–2 cm long and 3–4 storage roots 4–5 cm long.

Regeneration method

Asparagus plants can be regenerated through organogenesis or somatic embryogenesis. Organogenesis can be obtained either through direct differentiation of shoot buds from explants or through callus formation in explants and subsequent formation of shoots and roots [Hicks 1980]. In organogenesis pathway, callus can be induced from anther as well. For instance, Shen et al. [1995] studied the methods to improve the callus and plantlet formation rate from anther culture of asparagus. Anthers cultured on 1/2 Murashige and Skoog medium (MS) [Murashige and Skoog 1962] medium supplemented with 1.0 $mg \cdot L^{-1}$ 2, 4-D, 1.0 $mg \cdot L^{-1}$ NAA and 0.5 $mg \cdot L^{-1}$ BA was found to give the highest callus induction rate. And the organogenesis – cluster shoots were induced when calli were subcultured on MS medium with 0.1 $mg \cdot L^{-1}$ NAA and 0.1 $mg \cdot L^{-1}$ BA.

The differences between organogenesis and somatic embryogenesis were investigated by Bojnauth et al. [2003]. Through indirect organogenesis method, 4 mm nodal

explants from sterile seedlings were taken as the primary materials to be precultured on MS medium. Calli were induced successfully and they were observed to proliferate very quickly. Shoots and roots were well formed from these explants together with their primary calli.

Somatic embryogenesis involves the development of embryos from somatic cells and results in the production of complete somatic plantlet with the potential to grow into a whole plant. For somatic embryogenesis pathway, nodal explants treated on MS medium with 0.015 $mg \cdot L^{-1}$ NAA + 0.5 $mg \cdot L^{-1}$ BA possessed the highest plantlet regeneration rate (83.3%), but these plantlets were fragile and they needed to be subcultured repeatedly on pure MS over a 28 days period for two months. A complete plantlet with well developed cladophylls and storage roots was received through this method [Bojnauth et al. 2003].

Medium

Excessive callus and few, thin, weak roots may always be thorny problems in asparagus *in vitro* propagation. Components and properties of medium play pivotal roles in callus induction and plantlet rooting ability. Effects of plant growth regulators and culture methods, therefore, have been studied by scientists for decades (tab. 1).

Growth regulators. Growth retardants are able to modify the growth of plants when used exogenously [Grossmann et al. 1987]. Incorporation of growth retardants can promote the growth of strong shoots and roots of asparagus. Khunachak et al. [1987] investigated the effectiveness of some growth retardants including ancymidol, B-995, phosfon, Amo 1618, Cycocel and paclobutrazol (PP333). They found that growth retardants functioned differently, with ancymidol being the most effective. Similar effect of ancymidol on plantlet rooting ability was reported by Górecka et al. [1998]. They found that the highest rooting rate (30%) was observed from the plantlets cultured on the medium with ancymidol. The combinations of PP333, Indole-3-butyric acid (IBA), NAA and kinetin (KT) on MS medium were applied on axilary bud proliferation by Guo et al. [1993]. The use of PP333 gave slightly higher rooting rate than ancymidol. Through the study related to components integration for rooting, Wu et al. [2006] showed that additional growth retardant PP333 (1 $mg \cdot L^{-1}$) mixed with BA (0.5 $mg \cdot L^{-1}$) as well as KT (0.05 $mg \cdot L^{-1}$) plus 1/2 MS were the appropriate medium for rooting culture pretreatment. Newly, Lin et al. [2010] also found positive results of applying growth retardant – S3307. They concluded that S3307 could increase rooting rate in 0.1 $mg \cdot L^{-1}$ concentration at high temperature (25±1°C). They also stated that culture medium consisted of 1/2 MS + KT 0.1 $mg \cdot L^{-1}$ + NAA 0.1 $mg \cdot L^{-1}$ + indoleacetic acid (IAA) 0.5 $mg \cdot L^{-1}$ + IBA 20 $mg \cdot L^{-1}$ + S3307 0.1 $mg \cdot L^{-1}$ could optimize the rooting ability.

Some researchers illustrated that subculture [Shen et al. 2006] or multiculture or additional gibberellic acid (GA3) inhibitor [Chen and Zhou 1994] could effectively increase rooting rate. It can't be ignored that a group of artificially synthesized plant growth substances, e.g. IAA, IBA, NAA, 2,4-D, influences rooting in accordance with their combinations and concentrations. So far, in most of the cases studied, BA and NAA were applied to the primary culture medium for callus induction. Neither of these two substances was not higher than 2 $mg \cdot L^{-1}$ [Inagaki et al. 1981; Lai et al. 1991; Chen

et al. 1997; Bojnauth et al. 2003; Lin et al. 2008]. Sometimes 2, 4-D and KT were utilized for callus induction as well [Pontaroli and Camadro 2005; Saharan 2010]. Combined plant growth regulators in different concentrations were always added in order to optimize proliferation ability and potential organ regeneration capacity. For instance, asparagus shoot tips subculture experiment conducted by Shi et al. [1995] contained two steps. At first, shoot tips were cultured on 1/2 MS + 0.05 mg·L^{-1} KT + 0.5 mg·L^{-1} NAA medium for 7 days. Later on, they were incubated on medium consisted of 1/2 MS + 0.1 mg·L^{-1} KT + 1 mg·L^{-1} MET + 0.1% active carbon (AC). Through this way of culture, 80% rooting rate was received after 23 days of incubation.

In some studies, either NAA or IAA or IBA was applied to investigate the asparagus rooting ability. Results given by Wang et al. [2010] showed that asparagus rooting ability had the greatest sensitivity to NAA at 0.2 mg·L^{-1} concentration, through which 35.5% rooting rate was obtained. But in this way a big quantity of calli was received as well, which negatively affected to the growth of roots. On the contrary, 0.5 mg·L^{-1} IBA was more beneficial to the growth of roots.

The impact of GA3 inhibitor was also investigated in asparagus rooting culture. Saponins, the amphiphilic high molecular mass glycosides, were hypothesized to control delivery system of GA3 in asparagus shoot elongation in tissue culture. Study conducted by Saharan [2010] demonstrated that the optimal saponins concentration for shoot elongation (9.0 cm) and rooting rate (100%) was 3 mg·L^{-1} with 0.2 mg·L^{-1} GA3. However, with the same concentration of GA3, the rooting rate reduced from 100% to 55% by increasing the saponins concentration from 3 to 5 mg·L^{-1}.

Carbon resources. The influence of sugar on asparagus *in vitro* culture was reported in some studies [Harada and Yakuwa 1983; Shigeta et al. 1996; Wu et al. 2006]. Various kinds of soluble sugars were applicable. Either sucrose or glucose or fructose was usually the most frequently used one. Harada and Yakuwa [1983] demonstrated that the concentrations of sugars appropriate for root formation were higher (4%) than those for shoot formation (2%). Sucrose had more distinct effect on rooting ability in comparison to glucose and fructose. Lower than 3% sucrose was usually applied to callus initiation medium [Inagaki et al. 1981; Mamiya and Sakamoto 2000; Bojnauth et al. 2003; Pontaroli and Camadro 2005]. But sometimes higher concentration, e.g. 6%, was used as well [Lai et al. 1991; Gonzalez Castanon 2002]. A rooting medium containing high concentration of sucrose was reported to facilitate the development of plants [Conner et al. 1990]. Results reported by Górecka et al. [1998] and Gebler [2005] were alike too. In all breeding lines, 100% of rooted plantlets grown on the rooting medium with 30 g·L^{-1} sucrose could adapt to the greenhouse conditions [Górecka et al. 1998]. After applying different concentrations of sucrose to the rooting medium, Gebler [2005] found that plantlets formed storage roots on the medium containing 30 g·L^{-1} sucrose, whereas, they failed to develop the roots on the medium containing 6 g·L^{-1} sucrose. A good rooting process could be achieved by using different concentrations of sucrose [Mamiya and Sakamoto 2000]. In their study, somatic embryos were suggested to be firstly precultured in MS media containing 30 g·L^{-1} sucrose for 1 week and subsequently transferred to MS media containing 50 g·L^{-1} sucrose for 2 weeks. Through this way, over 50 mg roots were produced per plant.

Table 1. Studies on asparagus rooting *in vitro* in the years 1981–2010
Tabela 1. Badania nad ukorzenianiem szparaga *in vitro* w latach 1981–2010

Proliferation method Metoda rozmnażania	Callus induction medium Pożywka do tworzenia kalusa	Culture condition Warunki kultury	Explant material Eksplantaty	Proliferation and rooting medium Pożywka do namnażania i ukorzeniania	Culture condition Warunki kultury	Results Wyniki	Reference Literatura
Anther culture (Androgenesis)	MS + 0.7% agar + 1.0 mg·L^{-1} BA + 1.0 mg·L^{-1} NAA + 20 g·L^{-1} sucrose	25-27°C, 4000 lx, 16 hours per day	Callus derived from anther	MS + 0.7% agar + 2% sucrose + 1.0 mg·L^{-1} BA + 0.5 mg·L^{-1} NAA	25–27°C, 4000 lx, 16 hours per day	32% shooting rate was got. High ratio of BA relative to NAA was beneficial to shoot formation but not to root formation.	Inagaki et al. (1981)
				MS + 0.7% agar + 2% glucose + 0.1 mg·L^{-1} IBA	25–27°C, 4000 lx, 16 hours per day	After 14 weeks incubation, more than 45% rooting rate was obtained, but no shoots were formed.	
				MS + 0.7% agar + 2% glucose	25–27°C, 4000 lx, 16 hours per day	The best normal root elongation result (>30 mm) was obtained.	
				MS + 0.7% agar + 2% glucose + 1.0 mg·L^{-1} BA + 0.5 mg·L^{-1} NAA + 0.01 mg·L^{-1} GA_3	25–27°C, 4000 lx, 16 hours per day	Shoot formation rate was 10% less than that without GA_3. No roots were formed. GA_3 inhibited the formation and elongation of roots and shoots.	
Anther culture (Androgenesis)	1/2MS + 2 mg·L^{-1} NAA + 1 mg·L^{-1} BA + 6% sucrose	27 ± 1°C, in dark	Callus derived from anther	Firstly: Murachige et al. (1972) medium + 1 mg·L^{-1} NAA + 0.5 mg·L^{-1} BA + 2.5% sucrose (AR-2); two months later: MS + 3% sucrose.	27 ± 1°C, 1500 lx, 16 hours per day	Green plants formation rate was 3 times higher in MS basal medium than in AR-2 medium. The organ regeneration process of anther-derived callus from cv. UC500 W was well-established through multiculturing in AR-2 and MS media	Lai et al. (1991)

Somatic embryogenesis	MS + 30 g·L^{-1} sucrose + 1 g·L^{-1} 2-(N-morpholino) ethane-sulfonic acid + 4 g·L^{-1} gelrite	25°C, 3300 lx light, 16 hours per day	Somatic embryos	MS + 30 g·L^{-1} sucrose for 1 week and MS + 50 g·L^{-1} sucrose for 2 weeks.	27°C, 7400 lx cool white fluorescent light, 16 hours per day	Fresh weight of roots was over 50 mg per plant.	Mamiya and Sakamoto (2000)
Micro-propagation from explants	MS + 0.9% agar + 3% sucrose + 1 mg·L^{-1} KT + 1.5 mg·L^{-1} 2,4-D	26°C for 60 days in dark	2 mm long internodal spear sections	MS + 0.8% agar + 0.1 mg/l KT + 0.1 mg/l NAA + 1.32 mg/l ancymidol + 7% glucose	26°C in darkness	After 45 days, 100% rooting rate was achieved in cv. Argenteuil clone 265	Pontaroli and Camadro (2005)
Micro-propagation from explants	1/2MS + 2 mg·L^{-1} NAA + 1 mg·L^{-1} BA + 60 g·L^{-1} sucrose	not described	3 mm shoot tips	1/2MS + 0.1 mg·L^{-1} KT + 0.1 mg·L^{-1} NAA + 0.5 mg·L^{-1} IAA + 2.0 mg·L^{-1} IBA + 0.1 mg·L^{-1} S3307	25 ± 1°C, 2500 lx, 12 hours per day	After 30 days, rooting rate was 86.6%	Lin et al. (2008)
Micro-propagation from explants	MS + 0.5 mg·L^{-1} BA + 3% sucrose + 0.55% agar + 1.0 mg·L^{-1} NAA	not described	Lateral bud from second generation	First: MS + 0.02 mg·L^{-1} BA + 1.0 mg·L^{-1} PP333 + 0.2 mg·L^{-1} NAA + 3% sucrose + 0.55% agar; 15 days later: MS + 0.02 mg·L^{-1} BA + 3% sucrose + 0.55% agar + 0.5 mg·L^{-1} IBA	not described	Rooting rate was 53.3%.	Wang et al. (2010)
Micro-propagation from explants	MS + 1.0 mg·L^{-1} 2,4-D	not described	3 cm microshoots	MS + saponins 3 mg·L^{-1} + 0.2 mg·L^{-1} GA_3	not described	Shoot length reached 9.0 cm and rooting rate was 100%.	Saharan (2010)

Culture conditions

A successful *in vitro* culture process also depends on appropriate culture conditions. Temperature is a critical factor for callus induction and subsequent rooting culture. In many cases, 25 ± 2°C was always recommended for callus induction [Inagaki et al. 1981; Khunachak et al. 1987; Feng and Wolyn 1991; Chen et al. 1997; Lin et al. 2006a]. However, high temperature (≥25 ± 1°C) was not favorable for rooting. The optimal temperature for rooting was 20 ± 1°C [Lin et al. 2006b]. During *in vitro* culture, light condition is no less important than temperature as well. It affects formation of cladophylls which are responsible for photosynthesis. In the initial callus induction culture, light is not always needed. For instance, Feng and Wolyn [1991], Gonzalez Castanon [2002] as well as Pontaroli and Camadro [2005] reported their callus induction cultures were conducted in darkness. On the contrary, some other researchers illustrated different light intensity was applied during callus induction culture. It usually ranged from 1000 lx to 5000 lx [Inagaki et al. 1981, Lai et al. 1991, Chen et al. 1997, Mamiya and Sakamoto 2000]. Photoperiod together with light intensity influences the efficiency of organ formation, e.g. 16 hours light period was frequently applied in many plantlet proliferation cultures [Gonzalez Castanon 2002, Bojnauth et al. 2003].

Multiculture process

Callus age, plantlet age and pretreatment duration on the medium containing growth regulators were proved to give different rooting abilities. Pontaroli and Camadro [2005] applied 18-month-old and approximately 20-month-old calluses for two experiments. The latter one gave better plant regeneration ability. Yang and Clore [1974] investigated the effect of plantlet age on the rooting rate. They found that plantlets older than 4 weeks (8, 12, 16 and 20 weeks) could have more rooting percentage (>75%) after subculturing in MS + 0.1 ppm NAA+ KT for 2 weeks or more. A study conducted by Wu et al. [2006] clarified that when shoot tips were pretreated in MS + 0.5 $mg \cdot L^{-1}$ BA + 0.05 $mg \cdot L^{-1}$ KT + 1 $mg \cdot L^{-1}$ PP333 + 3% sucrose + 0.8% agar for 12 days before subculture in MS basal medium for 10 days, rooting rate peaked to 80%. Extending the pretreatment time did not increase rooting rate any more. Hence, 12 days pretreatment culture was considered to be beneficial for plantlet rooting.

Trends towards developing asparagus in vitro rooting technique

Firstly, aiming at establishing a long-term used and effective *in vitro* culture system in asparagus, attention must be paid to adjust the interactions between asparagus genotypes and culture medium. Because in some cases, even though the same culture medium was given, plantlets root induction abilities still varied due to different genotypes [Pontaroli and Camadro 2005].

Secondly, in order to obtain normal haploid plant, the selection of growth regulators and their ratio become critical. Since regenerated plant derived from anther culture is sometimes tetraploid or mixploid besides haploid and diploid. It happened probably during the anther *in vitro* culture, callus induced medium and differentiation medium contained many kinds of plant growth regulators, which led to cells unusual mitosis,

endoreduplication, nuclear fusion, appearance of polyarch spindle or chromosome non-disjuction [Lin et al. 2010]. Besides, the proportion of growth regulators strongly influences the formation of normal and abnormal roots. Plantlets with abnormal roots are always characterized by no primary roots, primary roots cracked, split primary roots, stubby primary roots with no secondary roots and even roots missing.

Improving plantlet survival rate and the proper transplant substrates selection are keys for plantlets development in the greenhouse and even their potential yield achievement. Chen [2007] demonstrated that if regenerated plantlets were trans-cultured in hormone free MS medium after in rooting induction medium, rooting rate, root length as well as stem height would have higher values. And after attempting different substrates, he found that substrates containing only coco shell powder gave the highest transplantation survival rate (95.2%) under greenhouse conditions (21 ± 2°C, 80–90% relative humidity). At the end, it must be emphasized that in order to obtain high quality transplantable plantlets from *in vitro* culture, multiculture for rooting and plantlet development is necessary.

At present, plant *in vitro* culture in sugar free medium has become a new tendency [Zeng 2005; Yang et al. 2007]. Due to the photoautotroph ability of plantlets inside the culture bottle, adjusting the compounds of culture medium in terms of removing sugars and increasing CO_2 concentration inside the bottle, can benefit the plantlet growth under closer natural condition as well as improve the survival rate of plantlets [Yang et al. 2007]. Applying new type of light resource, e.g. cold cathode fluorescent lamp (CCFL) [Tanaka et al. 2009] or field emission lamp (FEL) [Hamada et al. 2011] can also facilitate and improve the growth of plantlets. Thus, regulating CO_2 concentration, applying new type of light and altering culture medium compositions should be taken into consideration for the further development of asparagus *in vitro* culture technique.

REFERENCES

Bojnauth G., Puchooa S., Bahorun T., 2003. *In vitro* regeneration of *Asparagus officinalis*: preliminary results. Food and Agriculture Research Council, Reduit, Mauritius, AMAS, 7–15.

Cai K.X., Lin Z.K., Luo J.S., Gao H.C., Chen Z.D., Huang G.D., 2005. Factors affecting proliferation coefficient in *Asparagus officinalis* L. tissue culture. Fujian Sci. Technol. Tropical Crops. 30 (3), 6–7 [in Chinese].

Chen G.Y., Conner A.J., Christey M.C., Fautrier A.G., Field R.J., 1997. Culture and regeneration of protoplasts from shoots of asparagus cultures. Internat. J. Plant Sci. 158 (5), 543–551.

Chen W.F., Zhou X., 1994. Effects of GA_3 and S3307 on root formation in *Asparagus officinalis*. Plant Physiol. Communicat. 30 (1), 26–28 [in Chinese].

Chen Z.D., 2007. Studies of technology systems for tissue culture and rapid propagation of *Asparagus offcinalls* L. Southwest China J. Agric. Sci. 20 (3), 470–473 [in Chinese with English summary].

Conner A.J., Abernethy D.J., Falloon P.G., 1990. Transplantation of micropropagated asparagus plants to greenhouse conditions: the importance of *in vitro* root development. Acta Hort. 271, 128.

Doré C., 1977. *In vitro* techniques as an efficient tool in asparagus breeding. Acta Hort. 78, 89–93.

Falloon P.G., 1982. The need for asparagus breeding in New Zealand. New Zealand J. Exp. Agric. 10 (1), 101–109.

Falloon P.G., Nikoloff A.S., 1986. Asparagus: value of individual plant yield and fern characteristics as selection criteria. New Zealand J. Exp. Agric. 14 (4), 417–420.

Feng X.R., Wolyn D.J., 1991. High frequency of haploid embryos in asparagus anther culture. Plant Cell Rep. 10, 574–578.

Fortes G., Muller N., Augustin E., Silva J., Peters J., Flores R., 1997. The influence of spear number on *in vitro* rooting of asparagus (*Asparagus officinalis* L.). HortScience. 32 (3), 471.

Gebler P., 2005. Method development of obtaining supermale asparagus (*Asparagus officinalis* L.). PhD dissertation, Poznań University of Life Sciences, Poland, 1–84 [in Polish].

Gonzalez Castanon M.L., 1990. Evaluation of male and female asparagus plants. Interest in obtaining male or dioecious hybrids. Acta Hort. 271, 83–89.

Gonzalez Castanon M.L., 2002. Asparagus anther culture. Influence of genotype and growth environment conditions of the donor plants on the androgenetic response. Acta Hort. 589, 211–215.

Górecka K., Krzyżanowska D., Górecki R., 1998. The effect of different factors on asparagus rooting *in vitro* and adaptation. Materiały konferencji i obrad sekcji 51 Zjazdu PTB, Gdańsk, 167 [in Polish].

Grossmann K., Kwiatkowski J., Siebecker H., Jung J., 1987. Regulation of plant morphology by growth retardants. Plant Physiol. 84, 1018–1021.

Guo C.H., Ma F.T., Zhang Q.R., Li M.X., 1993. Root induction of asparagus *in vitro* by PP333. J. Northwest Sci-Tech Univ. Agric. Forestry. 21 (3), 32–36 [in Chinese with English summary].

Hamada K., Shimasaki K., Nishimura Y., Sasaoka H., Nishimura K., 2011. Applicability of a new developed field emission lamp for proliferation of Cymbidium PLB *in vitro*. Acta Hort. 907, 385–388.

Harada T., Yakuwa T., 1983. Studies on the morphogenesis of asparagus: VI. Effect of sugar on callus and organ formation in the *in vitro* culture of shoot segments of the seedlings. J. Faculty Agric., Hokkaido University. 61 (3), 307–314.

Hicks G.S., 1980. Patterns of organ development in plant tissue culture and the problem of organ determination. Botanical Review. 46 (1), 1–23.

Inagaki N., Harada T., Yakuwa T., 1981. Studies on the anther culture of horticultural crops: IV. Effect of growth regulators on organ formation from anther derived callus of *Asparagus officinalis* L. J. Faculty Agric., Hokkaido University. 60 (3), 236–249.

Inagaki N., Harada T., Yakuwa T., 1983. Studies on the anther culture of horticultural crops VII: Investigation of optimal conditions for obtaining pollen-originating callus and its characteristics in *Asparagus officinalis* L. J. Faculty Agric., Hokkaido University. 61 (3), 279–284.

Khunachak A., Chin C., Le T., Gianfagna T., 1987. Promotion of asparagus shoot and root growth by growth retardants. Plant Cell, Tissue and Organ Culture. 11, 97–110.

Kohmura H., Chokyu S., Harada T., 1994. An effective micropropagation system using embryogenic calli induced from bud cluster in *Asparagus officinalis* L. J. Jap. Society Hortic. Sci., 63 (1), 51–59.

Krzyżanowska D., Górecka K., Glapś T., Górecki R., Knaflewski M., 1995. Effect of genotypes on the asparagus regeneration and rooting *in vitro*. V Zjazd Hod. Rośl. Ogrod. Skierniewice 23–24. 2, 573–579 [in Polish].

Lai P.C., Hsu J.Y., Yeh C.C., Tsay H.S., 1991. Breeding for all-male plants through anther culture in asparagus: I. The anther culture efficiency among different varieties and plants. J. Agric. Res. China. 40 (1), 94–101 [in Chinese with English summary].

Lin Z.K., Luo J.S., Cai K.X., Chen Z.D., Gao H.C., Huang D.G., 2006a. Study on enrichment culture of *Asparagus officinalis* L. cespitose buds. Subtropical Plant Sci. 35 (1), 29–32 [in Chinese with English summary].

Lin Z.K., Gao H.C., Chen Z.D., Luo J.S., Cai K.X., Huang D.G., 2006b. Effects of several factors on rooting of *Asparagus officinalis* L. test-tube seedling. Plant Physiol. Communicat. 42 (4), 617–620 [in Chinese with English summary].

Lin Z.K., Zhang T.X., Yang J.J., Cai K.X., Liu C.Q., 2007. Study on callus inducement and green shoot differentiation of white asparagus anther. Chinese Agric. Sci. Bull. 23(9), 129–133 [in Chinese with English summary].

Lin Z.K., Chen Z.D., Cai K.X., Yang J.J., Zhang T.X., 2008. A study on the effects of several plant growth regulators on rooting of white asparagus (*Asparagus officinalis* L.) test-tube seedling from anther culture. Acta Agriculturae Univ. Jiangxiensis. 30 (4), 651–655 [in Chinese with English summary].

Lin Z.K., Wen Q.F., Lin Y.Z., 2010. Technology system of culture for *Asparagus offcinalis* L. anther. Fujian J. Agric. Sci. 25 (2), 212–216 [in Chinese with English summary].

Mamiya K., Sakamoto Y., 2000. Effects of sugar concentration and strength of basal medium on conversion of somatic embryos in *Asparagus officinalis* L. Sci. Hortic. 84 (1–2), 15–26.

Murashige T., Skoog F., 1962. A revised medium for rapid growth and bio assays with tobacco cultures. Physiol. Plant. 15 (3), 473–497.

Murashige T.M., Shabed M.N., Hasegawa P.M., Takatori F.H., Jones J.B., 1972. Propagation of asparagus through shoot apex culture. I. Nutrient medium for formation of plantlets. J. Am. Soc. Hortic. Sci. 97 (2), 158–161.

Pontaroli A.C., Camadro E.L., 2005. Plant regeneration after long term callus culture in clones of *Asparagus officinalis* L. Biocell. 29 (3), 313–317.

Reuther G., 1983. Application of tissue culture methods for improvement of *Asparagus officinalis* L. Asparagus Res. Newslett. 1, 2, 42–59.

Saharan V., 2010. Effect of gibberellic acid combined with saponin on shoot elongation of *Asparagus officinalis*. Biologia Plant. 54 (4), 740–742.

Shen S., Zou D., Zhang C., Liu S., 1995. Improved rate of callus and plantlet from anther culture of asparagus (*Asparagus officinalis* L). Acta Hort. 402, 299–305.

Shen H.L., Zhao N., Han Q.X., Dai F.W., 2006. Effects of stem-segment increased culture on rooting differentiation during quick reproduction of asparagus *in vitro*. Acta Agric, Borealo-Sinica. 21 (S1), 46–49 [in Chinese with English summary].

Shigeta J., Sato K., Tanaka S., Nakayama M., Mii M., 1996. Efficient plant regeneration of asparagus by inducing normal roots from *in vitro* multiplied shoot explants using gellan gum and glucose. Plant Sci. 113 (1), 99–104.

Shi K.W., Li S.Z., Zhang G.S., 1995. Effects of two-step rooting method on the rooting of asparagus (*Asparagus officinalis* L) by tissue culture. J. Zhejiang Agric. Sci. 2, 64–65 [in Chinese].

Tanaka M., Norikane A., Watanabe T., 2009. Cold cathode fluorescent lamps (CCFL): Revolutionary light source for plant micropropagation. Biotechnology & Biotechnological Equipment. 23 (4), 1497–1503.

Tang Y.P., Zhou J.S., Luo S.C., Sheng W.T., Xie Q.X., Zhu Y.L., Chen G.Y., 2011. A preliminary study on isolated microspore culture of *Asparagus officinalis* L. Acta Agric. Jiangxi. 23 (3), 14–16 [in Chinese with English summary].

Tsay H.S., Lai P.C., Chi N.C., 1980. Studies on anther culture and haploid plant regeneration of asparagus. J. Agric. Res. China. 29 (4), 309–319 [in Chinese with English summary].

Wang J.Y., Zhang X.P., Yang R., Li X.F., 2010. Effects of auxins on the propagation of Asparagus officinalis L. J. East China Normal Univ. (Nature Science). 6, 101–108 [in Chinese with English summary].
Wu C.R., Liu L.N., Yu A.P., Zhao X.M., Zhou G.Y., 2006. Different factors on formation of rooting in Asparagus officinalis in vitro. Bull. Botanic. Res. 26 (4), 430–434 [in Chinese with English summary].
Yakuwa T., Harada T., Tsuji H., 1982. Studies on the morphogenesis of asparagus: IV. The effect of transplanting on callus and organ formation of stem segment cultured *in vitro*. J. Faculty Agric., Hokkaido University. 61 (1), 151–159.
Yang H.J., Clore W.J., 1974. Development of complete plantlets from moderately vigorous shoots of stock plants of asparagus *in vitro*. HortScience. 9 (2), 138–140.
Yang Q.C., Liu W.K., Guan D.P., 2007. Design of device for fast propagation of plant sugar-free tissure and its environmental control system. J. Agric. Sci. Technol. 9 (4), 79–84. [in Chinese with English Summary].
Zeng B., 2005. Literature review of sugar-free technology in plant tissue culture. Nonwood Forest Res. 23 (2), 67–71.

CZYNNIKI WPŁYWAJĄCE NA ROZWÓJ KORZENI SZPARAGA (*Asparagus officinalis* L.) *IN VITRO*

Streszczenie. Linie rodzicielskie w hodowli szparaga są rozmnażane *in vitro*. Dodatkowo w celu otrzymania osobników supermęskich, niezbędnych do hodowli całkowicie męskich odmian, stosowane są kultury pylnikowe. Zdrowe i żywotne rośliny z kultur *in vitro* muszą posiadać dobrze wykształcone korzenie spichrzowe. Na ukorzenianie się szparaga *in vitro* wpływ ma wiele czynników. Tylko zdrowe eksplantaty, odpowiednia pożywka i warunki wzrostu umożliwiają otrzymanie roślin i ich dalszą uprawę w warunkach szklarniowych. W artykule zostały przedstawione trudności, czynniki, jak również tendencje w rozwoju techniki ukorzeniania szparaga *in vitro*.

Słowa kluczowe: szparag, ukorzenianie *in vitro*, regulatory wzrostu, pożywki

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