Effects of Different Explant Types, Plant Growth Regulators and Shoot Density on *In Vitro* Regeneration of Asparagus (*Asparagus officinallis* L.)

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Abstract

The asparagus plants known as Ka-nyut in Myanmar are commercially cultivated in many places of central lowlands and eastern mountainous regions. This experiment was carried out to observe the effects of different explants on *in vitro* shoot induction, to verify the optimum concentrations of 6-benzyladeno-purine (BAP) for shoot multiplication, and to compare the behaviours of the single shoots and cluster shoots for root induction of asparagus. Three different explant types – apical bud, lateral bud and spear segment – were cultured onto MS medium to induce shoots in initial culture. Shoot formation of three different explant types was observed around 20 days after culture. For initial culture, apical bud gave 63 % of shoot formation followed by lateral bud (44 %) and spear segment (20 %) respectively. Shoot tips derived from apical and lateral buds of initial culture were sub-cultured onto MS medium with 0.5 mg. L^{-1} of 1-naphthaleneacetic acid (NAA) and 0, 2, 3 or 4 mg. L^{-1} of BAP for shoot multiplication. The medium supplemented with 2 mg. L^{-1} of BAP gave the highest number of shoots (9 shoots per explant), the longest shoot length (2 cm), and the highest number of nodes (2 nodes per shoot). After shoot multiplication, two types of shoots (single and cluster shoots) were cultured again onto MS medium with 1.5 and 2.5 mg. L^{-1} of indole-3-butyric acid (IBA) for root induction. It was observed that cluster shoots formed roots well in 2.5 mg. L^{-1} IBA-containing medium. Cluster shoots have more potential for rooting of *in vitro* developing shoots than single shoots do.

Key words: shoot multiplication, root induction, asparagus

Introduction

Asparagus is a large genus with over 150 species of herbaceous perennial crops of high economic value in the Liliaceae family with a chromosome number of 2n=20. They are grown throughout the world although they originated mainly from Asia, Africa and Europe (Prohens et al. 2008). The most economically important Asparagus species is garden asparagus (*Asparagus officinallis* L.), which is a highly-prized vegetable. Tender and unexpanded shoots, commonly called spears, are the edible organs of garden asparagus. Asparagus growing has become popular in Myanmar. It is commercially cultivated in many places of upper Myanmar and in the central lowlands. Its commercial production is also initiated in many areas by private growers. Asparagus has been identified as having marketable value as a medicinal plant with residential and commercial applications. Green asparagus scores higher in micro-nutrients (Fe 1.5 mg, ascorbic acid 48 mg) than white asparagus.

Propagation of asparagus by seed or by division of individual crown are commonly used in Myanmar. However, propagation by seed results in a low percentage of germination and clonal propagation by division of individual crown is very slow as one

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plant gives only 2-4 new plants per year even under optimum conditions in the absence of any pest invasion of injured surface (Ornstrup 1997). As populations of asparagus seedlings are highly heterozygous, pure inbred homozygous lines are difficult to develop. Therefore, individual seeds harvested from the same plant normally have different genotypes – a variability that can adversely affect the yield of asparagus in production fields because the productivity of individual plants may differ markedly. Therefore, to avoid yield variation from the use of heterozygous seed lots in asparagus fields, micropropagation techniques have been used to multiply male genotypes as they produce higher yields than do the female and hermaphrodite ones (Desjardins and Bajai 1992).

In micropropagation technique, shoot and root induction can be affected by explant source, shoot density and plant growth regulators (Jianwu et al. 2012). The maximum rate of micropropagation depends on the selection of the most suitable explants (Murashige 1974). Lateral bud density on a solid medium can affect shoot and root growth of asparagus. The number of young shoots can influence on asparagus rooting (Fortes et al. 1997).

NAA and BAP or 6-benzyladenine (BA), auxin and cytokinin are commonly used in asparagus culture to regulate cell division, internode elongation and shoot differentiation respectively (Razdan 2003). Auxin plays a key role in rooting, and exogenous auxin is required in many species. In many commercial propagation systems of other species, however, IBA is most common (Deklerk et al. 1999). Experiments to optimize the kind and concentration of plant growth regulators will be critical in culture of asparagus. To our knowledge, there are no reports of *in vitro* culture of *Asparagus officinallis* L. in Myanmar. Thus, the aim of this experiment was to observe the effect of different explant types on *in vitro* shoot induction, the optimum concentrations of BAP for shoot multiplication, and to compare the single shoots and cluster shoots for root induction of asparagus.

Materials and Methods

The experiment was conducted at Plant Tissue Culture Laboratory of Department of Horticulture, Yezin Agricultural University, from September 2016 to August 2017.

Explant preparation: Fifteen- to twenty- centimeter – long healthy young spears emerging from two year old hybrid asparagus farm were harvested in the early morning. The basal (10 cm) regions of these spears were discarded and the remaining portions were used as experimental materials. The collected spears were washed in running water and surface-sterilized with commercial bleach (15 %) for 15 minutes and rinsed thoroughly in sterile distilled water 3-4 times. Apical buds, lateral buds and spear segments (5-7 mm in length) from sterilized spears were excised aseptically in a Laminar Air Flow Chamber and used as explants.

Culture medium and condition: The explants were inoculated onto MS medium (Murashige and Skoogs 1962). The pH of the medium was adjusted to 5.8 and autoclaved at 121 º C for 15 minutes. The molten media were dispensed 30 ml into 150 ml cornical flasks. Cultures were maintained in the transfer room at 25 ± 2ºC and the light intensity was 30 µ. mol. m^{-2}. s^{-1} at daily periods of 16 hours light.

Shoot induction: Three different explant types – apical buds, lateral buds and spear segments – were cultured onto MS medium. The experiment was set up in completely randomized design (CRD) with twelve replications. Number of shoots per explant, shoot length (cm) and number of nodes per shoot were recorded six weeks after culture.

Shoot multiplication: After six weeks, two different explant types developed from initial culture, i.e. shoot tips derived from apical and lateral bud cultures, were transferred onto plant growth regulators (PGRs) free medium for two weeks. And then, when they were 5-7 mm long, they were transferred onto MS medium supplemented with different concentrations of PGRs: BAP 0.0, 2.0, 3.0 and 4.0 mg. L^{-1} and NAA 0.5 mg. L^{-1} for shoot multiplication. The experiment was set up in factorial randomized complete block design (RCB) with twelve replications. Number of shoots per explant, shoot length (cm) and number of nodes per shoot were recorded eight weeks after culture.

Root induction: At eight weeks after shoot multiplication, shoots produced from shoot multiplication

stage (4-5 cm in length) were separated as cluster shoot and single shoot types. And then, two different type shoots were transferred onto MS medium with IBA (1.5 mg. L^{-1} and 2.5 mg. L^{-1}) for root induction. The experiment was set up in factorial randomized complete block design (RCB) with twelve replications. The data were recorded in terms of percentage of rooting.

Statistical analysis: The results were analyzed for statistical significance by using Statistix 8 (version 8.0) software. Means were compared with Least Significant Different (LSD=0.05).

Results and Discussion

Shoot induction: Shoot induction started within two weeks of culture in all explants. The effects of different explants on number of shoots per explant, shoot length and number of nodes per shoot were described in table 1. There was highly significant effect of explant types on number of shoots per explant, shoot length and number of nodes per shoot.

The maximum number of shoots, 2.7 per explant, was obtained from apical bud explants followed by spear segment explants with 1.3 shoots. The apical bud explants produced more number of shoots per explants than lateral bud and spear segment explants. It might be due to the different rates of cell division in different explant regions. Apical buds used as explant were located in the portion of spear tip, which is, in other words, the apical meristem and sub-apical meristem region. In asparagus, most cell division occurs in the apical meristem of spear tip (Culpupper and Moon 1939). Brown and Sommer (1992) reported that the apical meristem was responsible for most of the organogenesis phenomena normally associated with shoot morphogenesis.

The longest shoot length, 2.6 cm, was recorded from lateral bud explants, significantly higher than those of apical bud (1.8 cm) and spear segment (1.3 cm) explants. It might be due to the more active cell elongation zone in the lateral bud explant. Lateral buds used as explant were located beneath the apical and sub-apical meristem region. That is the main elongation zone in the asparagus spear and about 20 mm long (figure 1) (Kojima et al. 1993). Therefore, the shoots resulted from lateral bud explants were observed to be longer than those from apical bud and spear segment explants.

The maximum number of nodes, 2.4 per shoot, was obtained from lateral bud explants followed by apical bud explants (2.1) and spear segments explants (1.1). Differences in number of nodes per explant may be due to the different rates of cell divisions in the spear. The juvenility of spear seg-

Table 1. Effects of different explant types on number of shoots per explant, shoot length and number of nodes per shoot in shoot induction of asparagus

Explants	Number of shoots per explant	Shoot length (cm)	Number of nodes per shoot
Apical bud	**2.7 a**	1.8 b	**2.1 a**
Lateral bud	2.0 b	**2.6 a**	**2.4 a**
Spear segment	1.3 c	1.3 b	1.1 b
LSD (0.05)	0.62	0.73	0.69
CV %	69.67	88.04	85.03
Pr>F	**	**	**

Means within a column followed by the same letter are not significantly difference at 5 % LSD
* Significant at 5 % level, ** Significant at 1 % level

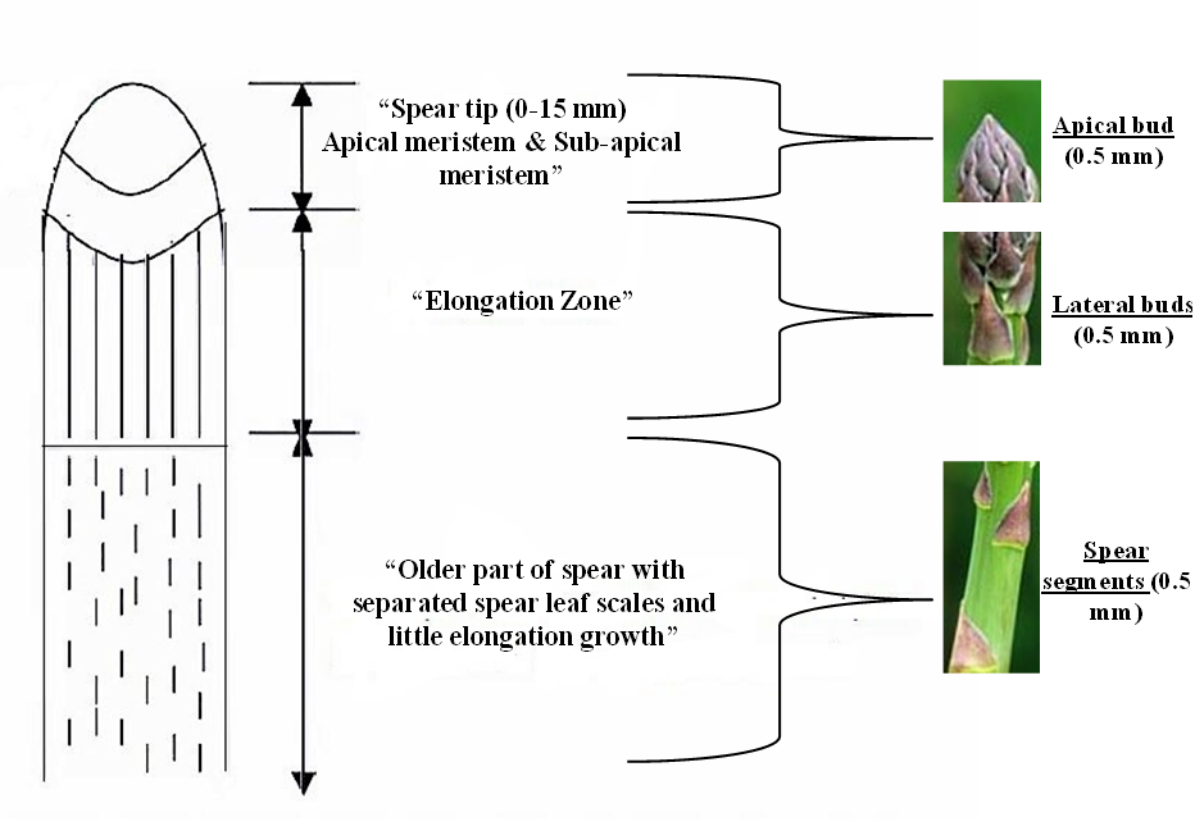

Figure 1. Diagrammatic representation of an asparagus spear

ments was lower than that of other two explants. That may be the reason why the number of nodes per shoot induced from spear segment explants was lower than those of apical and lateral bud explants. Therefore, spear segment is not suitable for *in vitro* propagation of asparagus.

Shoot multiplication: Shoot tips derived from apical and lateral bud cultures responded to the different BAP concentrations. At two weeks after culture, it was noticed that many of the explants started to grow. The effects of different explant types and concentrations of BAP on number of shoots per explant, shoot length and number of nodes per shoot were described in table 2. The effects of different explant types were not significantly different in number of shoots per explant, shoot length and number of nodes per shoot. However, the effects of different concentrations of BAP were significantly different in all these parameters.

The maximum number of shoots, 8.9 per explant, was obtained from 2 mg. L^{-1} BAP- containing media followed by 6.1 per explant obtained on 0 mg. L^{-1}- containing medium. Kamile et al. (2014) found that MS medium with 2 mg. L^{-1} BAP and 0.5 mg. L^{-1} NAA was the best propagation medium for *in vitro* multiplication of *Asparagus stipularis* Forssk. with 6.5 shoots per explant. The finding in this study agreed with the report of Strosse et al. (2008) that there was a decrease in survival percent with increasing PGRs concentrations. Therefore, the number of shoots per explant was found to increase when BAP concentrations was 2 mg. L^{-1} but decreased when BAP concentrations became more than 2 mg. L^{-1} BAP (Figure 2).

The longest shoot length, 2.1 cm, was obtained from 2 mg. L^{-1} BAP- containing media. The shortest shoot length, 1.2 cm, was recorded from 4 mg. L^{-1} BAP- containing media. The decrease in shoot length might be due to the effect of higher concentrations of BAP (Paek and Hahn 2000).

The maximum number of nodes, 2.2 per shoot, was obtained from 2 mg. L^{-1} BAP- containing media followed by 1.7 from 0 mg. L^{-1} BAP- containing

Table 2. Effects of different explant types and BAP concentrations on number of shoots per explant, shoot length and number of nodes per shoot in shoot multiplication of asparagus

Treatment	Number of shoots per explant	Shoot length (cm)	Number of nodes per shoot
Explant types			
Shoot tips derived from apical bud culture	**5.5 a**	**1.7 a**	**1.8 a**
Shoot tips derived from lateral bud culture	**6.3 a**	**1.6 a**	**1.6 a**
LSD (0.05)	2.47	0.43	0.26
PGRs level			
0 mg.L^{-1} BAP	6.1 **ab**	1.9 **ab**	1.7 b
2 mg.L^{-1} BAP	8.9 **a**	2.1 **a**	2.2 **a**
3 mg.L^{-1} BAP	4.4 b	1.4 bc	1.5 b
4 mg.L^{-1} BAP	4.2 b	1.2 c	1.5 b
LSD (0.05)	3.49	0.61	0.37
Pr>F			
Explant type	ns	ns	ns
PGRs	*	*	**
Explant type × PGRs	ns	ns	ns
CV%	47.72	30.11	17.28

Means within a column followed by the same letter are not significantly difference at 5 % LSD
* Significant at 5 % level, ** Significant at 1 % level

media. It was found that 2 mg. L^{-1} BAP- containing media produced more number of nodes per shoot than did other treatments. This finding also confirmed the report of Armstrong and Razdon (2001) that some plant species have enough levels of endogenous hormones and do not require a high level of exogenous regulators for plant regeneration.

Root induction: Effects of shoot density and different concentrations of IBA on asparagus root formation were shown in figure 3. The maximum root induction (40 %) was found in cluster shoots on the medium containing 2.5 mg. L^{-1} IBA. Minimum root induction (4.44 %) was recorded in single shoots on medium containing 1.5 mg. L^{-1} IBA. It was observed that cluster shoots have higher root induction frequency than do the single shoots on both media. It might be due to the nature of the explant type.

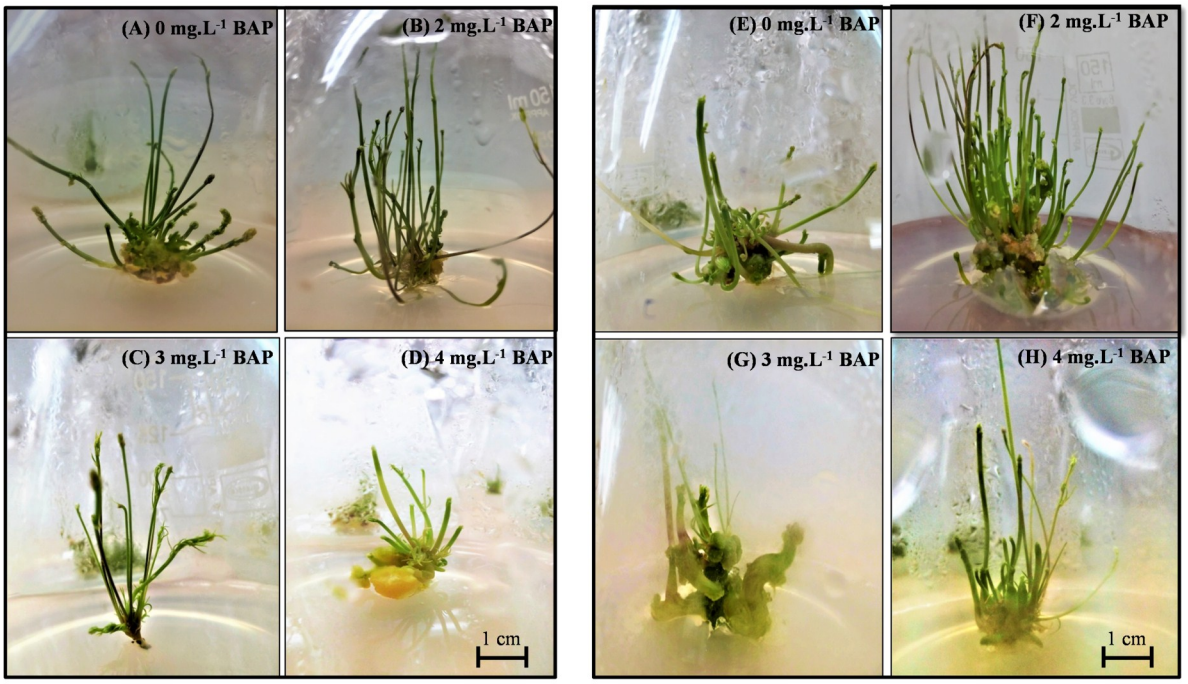

Figure 2. Shoot multiplication of shoot tips derived from apical buds (A, B, C, and D) and lateral buds (E, F, G and H) on media containing 0.5 mg. L^{-1} NAA with 0, 2, 3 and 4 mg. L^{-1} BAP respectively after six weeks culture

Shen et al. (1995) found that when both cluster shoots (with 2-5 shoots) and single shoots were cultured onto MS ½ medium with 0.1 mg. L^{-1} NAA, a higher rooting percentage (78.9 %) was observed in cluster shoots where only 25.7 % was recorded in single shoots. Mehta and Subramanian (2005) reported that roots were induced in Asparagus species on the media containing IBA among several PGRs.

Conclusions

Based on the findings, in shoot induction stage, results mentioned above revealed apical buds and lateral buds of asparagus spear had potential to produce plantlets through *in vitro* cloning. Moreover, it was found that explant type influenced its regeneration capability.

In shoot multiplication, BAP concentration plays a key role and it was noticeable that 2 mg. L^{-1} BAP was suitable. Higher BAP concentrations tend to produce abnormal shoots.

In root induction stage, not only IBA concentrations but also shoot density pointed out to be governing factors in root induction. Suitable concentration of IBA was needed to induce roots. Cluster shoots should be used for root induction culture.

To our knowledge, this was the first report for the protocol of *in vitro* culture of edible green asparagus in Myanmar. Direct organogenesis was attempted for the first time to obtain preliminary information on propagation of this plant. Further research is required to promote the asparagus multiplication by using a wide range of concentrations and combinations in plant growth regulators, different genotypes of asparagus species and concentrations of media components.

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