Pak. J. Bot., 55(3): 1035-1040, 2023. DOI: http://dx.doi.org/10.30848/PJB2023-3(24)

MORPHOLOGICAL CHARACTERIZATIONS OF BUD, ANTHER AND MICROSPORES IN NGOC LINH GINSENG - *PANAX VIETNAMENSIS* HA *ET* GRUSHV. VAR. *VIETNAMENSIS* (ARALIACEAE)

TU XUAN DINH[1], TIEN TON NU BAO HUYEN[2] AND LY MINH NGUYEN[2*]

[1]*Incubation and Support Center for Technology and Science Enterprises, Vietnam*
[2]*Faculty of Biology and Environmental Science, The University of Danang. University of Science and Education, Vietnam*
[*]*Corresponding author's email: nmly@ued.udn.vn*

Abstract

Ngoc Linh ginseng (*Panax vietnamensis* Ha *et* Grushv. var. *vietnamensis*) is a valuable and endemic medicinal plant of Vietnam. Propagation of Ngoc Linh ginseng plants is mainly dependent on seeds produced via self-fertilization. However, little is known about reproduction development in Ngoc Linh ginseng. In this study, several morphological characteristics of inflorescences, floral buds, anthers, and microspores were observed and described. The results showed that floral buds ranged from 0.5 to 4.4 mm, anthers from 0.48±0.28 to 1.56±0.45 mm, and microspores from 20.82±2.49 to 41.22 ± 1.44 µm. A strong correlation between bud length, anther length and developmental stage of microspores were shown, which were 0.905 and 0.781, respectively. Moreover, microspores of different developmental stages in a single anther were observed. These study results can be used to improve the sexual reproduction of this ginseng.

Key words: Anther, Floral buds, Inflorescence, Microsporogenesis, *Panax vietnamensis*.

Introduction

Ngoc Linh ginseng (*Panax vietnamensis* Ha *et* Grushv. var. *vietnamensis*) belongs to the Ginseng genus (*Panax*) of the Araliaceae family. It yields a leading medicine in precious traditional medicine in Vietnam. According to many studies, Ngoc Linh ginseng root contains up to 52 types of saponins, including many new compounds such as majonoside R2 (MR2), ginsenoside Rh5 (20-O-Me-G-Rh1), vina-ginsenoside R1-R25 (Nguyen *et al.*, 2018; Nguyen & Phuong, 2019). The compounds were found to exhibit liver protective, anti-cancer, anti-oxidant, and antistress properties (Nham *et al.*, 1995; Huong *et al*., 1998; Konoshima *et al.*, 1998; Yobimoto *et al.*, 2000; Tran *et al.*, 2002).

Currently, there is a massive demand for this ginseng species in Vietnam. However, the overexploitation status has been maintained in recent years while the reproductive capacity of this ginseng species is low, leading to the danger of extinction (Vietnam Red Data Book). Therefore, an urgent issue is to conserve and increase the genetic diversity of this ginseng. Previous studies have focused on *In vitro* propagation techniques of Ngoc Linh ginseng (Mai *et al.*, 2014; Vu *et al.*, 2014; Diem *et al*., 2022). However, few studies were also conducted on reproduction development in Ngoc Linh ginseng, such as the development of the male organ. In higher plants, male reproductive development is a complex biological process that includes stamen identity specification from the floral meristem, anther morphogenesis, and the formation of pollen grains via meiosis and mitosis within the flower (Wilson & Zhang, 2009).

Furthermore, morphological characteristics of floral buds, anthers, and microspores have different features in different species and determine the ability of pollination and the success of androgenesis to obtain doubled haploids in plants (Ibrahim *et al.*, 2014; Kim *et al.*, 2015; Nguyen *et al*., 2019). In this study, we described the unique features of inflorescences of Ngoc Linh ginseng. Mainly, we comprehensively investigated characteristics of floral buds, anther morphogenesis, and microspores in Ngoc Linh ginseng and the correlation between developmental stages of microspores and floral bud and anther length.

Materials and Methods

Fresh inflorescences and floral buds of 5-6-year-old cultivated Ngoc Linh ginseng were collected from the experimental station of the National Center for Research and Development of Vietnamese Ginseng in the Ngọc Linh mountain area of Kon Tum Province (14º58'34"N and 107º54'41"E).

Floral buds were collected and fixed in Carnoy solution (3 ethanol: 1 acetic acid) and refrigerated. The morphological characteristics of floral buds and their corresponding anthers were observed and determined by Zeiss Axio Lab1 stereomicroscope (Suzhou co., Ltd). The number and size of flowers were measured from 10 individual plants in each sampling time.

Microspores were isolated from the anther. The cell nucleus was stained with a fluorescent dye 4 ', 6-diamidino-2-phenylindole (DAPI), and observed under fluorescence microscope Zeiss Axio Lab1 (Suzhou co., Ltd) at 358 nm (Ferrie & Caswell, 2011). The growth stages of microspores were determined by the location and number of nuclei in the cell (Blackmore *et al*., 2007). One hundred microspores were randomly selected for evaluation with three replicates to determine the percentage of each stage in the same floral bud.

Statistically significant differences were tested using analysis of variance (ANOVA) and Tukey test with $\alpha = 0.05$. The data is statistically described and processed on statistical software IBM SPSS Statistics 22.

Results and Discussion

Single canopy inflorescences contained floral buds being green throughout the development process until opening (Fig. 1). Diameter values of these inflorescences ranged from 0.7-1.3 cm with a large number of floral buds, about 94.7±6.3 buds. This result was similar to those in studies of Lai Chau ginseng (*Panax vietnamensis* var. *fuscidiscus* K. Komatsu, S. Zhu & S.Q. Cai) (Zhu *et al*., 2003), yet the figure was higher than some other ginseng species (Carpenter & Cottam, 1981; Schlessman, 1985). As to American ginseng (*P. quinquefolium*), the number of floral buds per inflorescence depended on morphological class (number of leaf/prong), with a higher number of prongs being observed in older plants. This number could reach a peak of 47.3±6.1 floral buds (Lewis & Zenger, 1982). The number of flowering plants forming fruit accounted for 26.9±4.1 percent of all flowering plants, and the average number of fruit was only 3.5±2.5 per plant. This proportion was relatively low compared to other ginsengs (Lewis & Zenger, 1983). The peduncle length, which fluctuated around 16.4±2.0 cm, was similar to Korean ginseng (Kim *et al.*, 2015).

The flowering began with the outermost edge of the umbel and proceeded inwards (Fig. 1 A1). The duration of flowering of Ngoc Linh ginseng inflorescence, roughly 32.8±7.0 days, sometimes up to 40 days, was longer than that of American ginseng (Schluter & Punja, 2000).

Besides, there was a positive correlation between the number of flowers per inflorescence and flowering duration (r = 0.535). This pattern was also observed in other ginseng species (Schluter & Punja, 2000).

Features Ngoc Linh floral buds and anthers: The flower of Ngoc Linh ginseng was morphologically bisexual, with five sepals, five petals, five stamens, two equal-sized anthers hanging on each stamen, and one or two (or rarely three) styles. One-styled flower accounted for 85.6±12.1 percent of all flowers. This observation was similar to American ginseng (Schlessman, 1985). At the initial stage, the immature floral buds were green, both petals and sepals were unopened (Fig. 2 B1).

During the growth, the individual petals became separated, with white visible at the apical region (Fig. 1 A2, circle). As the floral buds began to open, the sepals gradually turned dark green, and the anthers began to expand (Fig. 2 B4). This development was also recorded in *Panax ginseng* and *P. quinquefolius*. However, as to *P. quinquefolius,* buds were pale yellow at the initial developmental stage (Kim *et al.*, 2015).

The length of Ngoc Linh ginseng buds ranged from 0.5 to 4.4 mm. Flowers started opening with a minimum length of 3.1 mm (Table 1). Besides, an increase in the size of the anther and the size of the floral buds and flowers were noticed. The correlation coefficient between the anther length and floral buds was r = 0.881 with α = 0.01 (This result was not shown). Based on the difference in the size of the anther, the bud can be divided into eight different size ranges (Table 1).

The anther of floral buds had the smallest length of 0.48±0.28 mm, and the largest reached 1.56±0.451 mm. The anthers of the blossoming flower ranged from 1.16±0.07 to 1.56±0.45 mm. During floral bud development, the color of the floral bud did not change. However, the anthers gradually changed from green to greenish-yellow and white (Fig. 2B6, B7, and B8). When the buds reached a size of 3.1 mm, the anthers protruded from the buds, and pollen grains appeared on the surface of the anthers (Fig. 1 A2), while only the stamens of *P. ginseng* remained white and unchanged (Kim *et al*., 2016). This result showed that Ngoc Linh ginseng pollen had begun to ripen and could be used to pollinate from this stage.

Identifying microspore developmental stages is essential for further fundamental research and applications, including anther culture or isolated microspore culture technique to regenerate double haploid plants in Ngoc Linh ginseng (Germana, 2011; Parra-Vega *et al.*, 2013; Mangal & Srivasatava, 2019). To better understand the development of floral buds in Ngoc Linh ginseng, cytological changes were observed and described.

In this study five microspore developmental stages were observed, including meiocytes, tetrad, early uninucleate, late uninucleate, and binucleate (Fig. 3). These stages were determined through the number and location of nuclei in the cell (Blackmore *et al.*, 2007). In the same bud size, different stages could be observed. However, there was a dominant developmental stage, which was similar to several other plant species (Sumarmi *et al.*, 2014; Nguyen *et al.*, 2019).

Table 1. Correlation between floral buds size and length of Ngoc Linh ginseng anthers.

No.	Floral bud length (mm)	Anther size (mm)	Microspore diameter (µm)	Morphology of floral bud	Morphology of anther
1.	0.5-1.0	0.48 ± 0.28^{a}	20.82 ± 2.49^{a}		
2.	1.1-1.5	0.83 ± 0.07^{b}	23.64 ± 1.07^{b}		
3.	1.6-2.0	0.93 ± 0.079^{bc}	24.40 ± 1.37^{bc}		
4.	2.1 -2.5	1.04 ± 0.07^{bcd}	25.10 ± 2.40^{cd}		
5.	2.6-3.0	1.11 ± 0.04^{cd}	25.63 ± 2.49^{de}		
6.	3.1-3.5	1.16 ± 0.07^{cd}	26.26 ± 2.62^{e}		
7.	3.6-4.0	1.27 ± 0.22^{d}	33.24 ± 3.46^{f}		
8.	4,1-4,4	1.56 ± 0.45^{e}	41.22 ± 1.44^{g}		

Note. The different letters on the same column indicate the difference in the statistical significance of the sample mean with $p<0.05$

Fig. 1. Ngoc Linh ginseng inflorescences. A1 – Picture of Ngoc Linh ginseng plants in a ginseng field. A2 – Morphology of the Ngoc Linh ginseng inflorescence under stereomicroscope with scale bar = 2.0 mm.

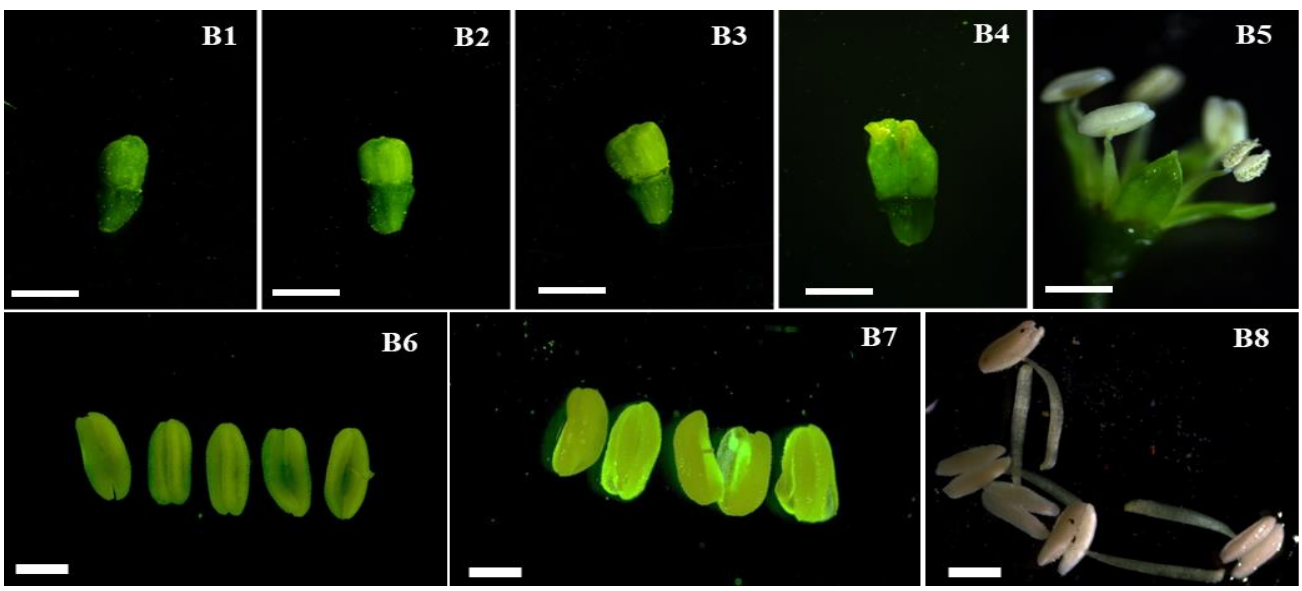

Fig. 2. The change of characteristics during the development stage of floral buds and flowers (A1 - A5) with scale bar = 1 mm, anthers (B1 - B3) with scale bar = 0.5 mm.

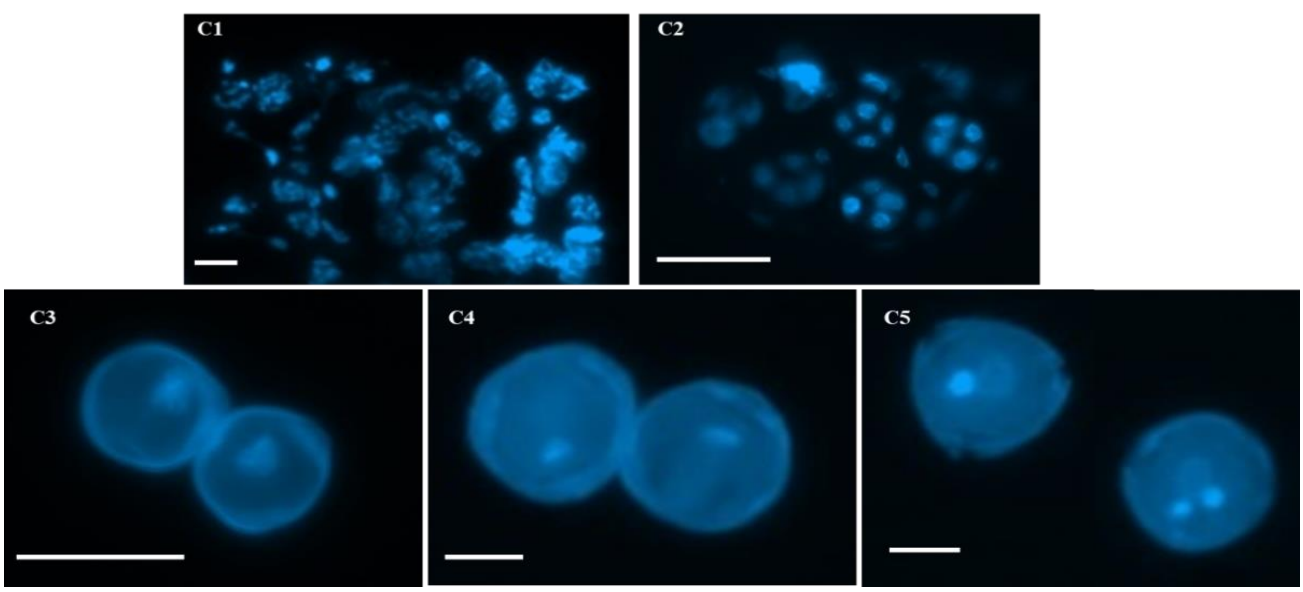

Fig. 3. Microspore developmental stages: C1 - Meiocyte; C2 - Tetrad; C3 - Early uninucleate; C4- Late uninucleate; C5 - Binucleate. Scale bar: 25 µm.

Table 2. The relationship between floral buds size and microspore developmental stages.

No.	Floral bud size (mm)	Microspore developmental stages (%)				
		Meiocyte	Tetrad	Early uninucleate	Late uninucleate	Binucleate
1.	0.5-1.0	95.19 ± 0.64	4.82 ± 0.64	0	0	0
2.	1.1-1.5	14.44 ± 3.33	61.11 ± 2.22	20.74 ± 1.69	3.70 ± 2.31	0
3.	1.6-2.0		26.67 ± 4.01	68.15 ± 4.63	5.19 ± 2.31	0
4.	2.1-2.5		7.04 ± 1.28	61.85 ± 4.49	18.15 ± 1.70	12.96 ± 3.90
5.	2.6-3.0		0	34.07 ± 1.70	45.93 ± 3.57	20.00 ± 4.01
6.	3.1-3.5		0	0	22.96 ± 1.70	77.04 ± 1.70
7.	3.6-4.0		0	0	12.22 ± 2.22	87.78 ± 2.22
8.	4,1-4,4		0	0	5.19 ± 2.31	94.82 ± 2.31

Note: Different letters on the same column indicate the difference in the statistical significance of the sample mean.

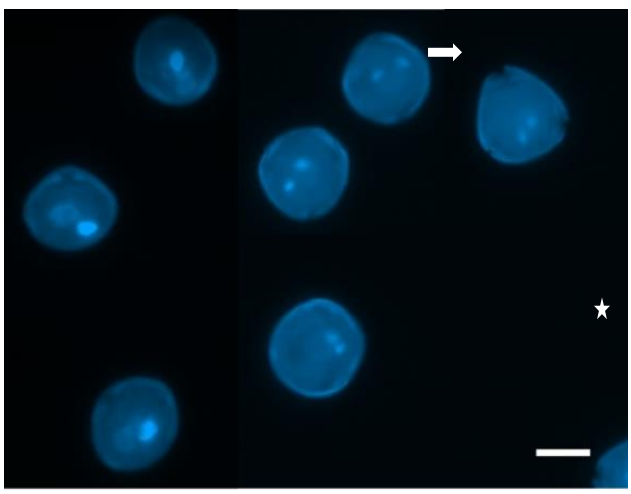

Fig. 4. Different developmental stages of microspores in anther. Early uninucleate stage (indicted as arrow), Binucleate stage (indicted as star). Scale bar = 25 µm.

Floral buds ranged from 0.5 to 1.0 mm with anthers covered in petals, containing cells at the meiocyte and tetrad stages (95.19% and 4.82%) without microspores (Table 2). During the tetrad phase, 4 separate microspores were still encapsulated within the cell wall (Fig. 3 C2), observed in closed floral buds (Fig. 2 B1). At this stage, the anthers were green (Fig. 2 B6).

The early uninucleate stage was observed in most bud sizes. In particular, this stage occupied the highest rate in floral buds of 1.5-2.0 mm (68.15%). Early uninucleate microspores (Fig. 3 C3) released from tetrad showed an irregular shape, thin cell walls, large nuclei, and average diameter at each bud size ranging from 23.64±1.07 µm to 25.63±1.70 µm. The buds remained in shape (Fig. 2 B2), while the anthers turned greenish-yellow (Fig. 2 B7).

The unopened floral buds were 2.1-2.5 mm with anthers containing microspores at the early uninucleate (61.85%) was the dominant stage. Late uninucleate microspores were spherical; the nucleus was skewed on one side of the cell, forming vacuoles, thick cell walls (Fig. 3 C4). At this stage, floral buds and anthers remained **these above features** (Table 1). Floral buds dominated the binucleate stage from 3.1 mm to 4.4 mm. This stage was characterized by a larger vegetative nucleus and a smaller reproductive nucleus (Fig. 3 C5).

The diameter of Ngoc Linh ginseng microspore ranged from 20.82±2.49 µm to 41.22±1.44 µm. Besides, based on the research results of Kim *et al*., (2016), the diameter of Ngoc Linh ginseng microspores was bigger than Korean ginseng (*Panax ginseng*); the maximum diameter of Korean ginseng was 27 µm. Different developmental stages of microspores could be observed in the same anther. The microspore shape could vary from a circle to an oval (Fig. 4).

In this study, several stages of development of Ngoc Linh ginseng microspores had been identified, and this result was similar to that of Kim *et al*., (2015) on Korean ginseng subjects. However, it was still possible to notice the main difference between these two species of floral bud size. Besides, Kim *et al*., (2015) did not give the percentage between different development stages of the same anther, a floral bud.

In some species, several morphological characteristics of floral buds and anthers were functional and visible markers for selecting buds with microspores at particular developmental stages (Lauxen *et al*., 2003; Adhikari & Kang, 2017). For most plants, the floral bud length is usually used as the most convenient and accurate criterion to select the microspore developmental stage (Sato *et al*., 2002; Han *et al*., 2014; Barroso *et al*., 2015). In sweet pepper, the combination of the ratio between calyx length and bud length (calyx/bud ratio) and anther pigmentation is an easy and fast predictor of individual microspore/pollen developmental stages (Parra-Vega *et al.,* 2013).

According to our results, the correlation coefficients between the developmental stage of microspores with floral bud and anther length were 0.905, 0.781 (Fig. 5). Therefore, both buds and anther length could be a reliable criterion to be used to determine the microspore developmental stage in Ngoc Linh ginseng.

Conclusion

In this study, we described morphological characteristics of **inflorescence**, floral buds, anthers, and microspores in Ngoc Linh ginseng (*Panax vietnamensis* Ha et Grushv.), and some features were different from other ginseng. Moreover, the correlations between floral bud size, anther size, and microspore developmental stages were assessed. This information can serve the research involved in sexual reproduction improvement as well as the breeding process of this endemic and endangered (likely to face extinction) ginseng.

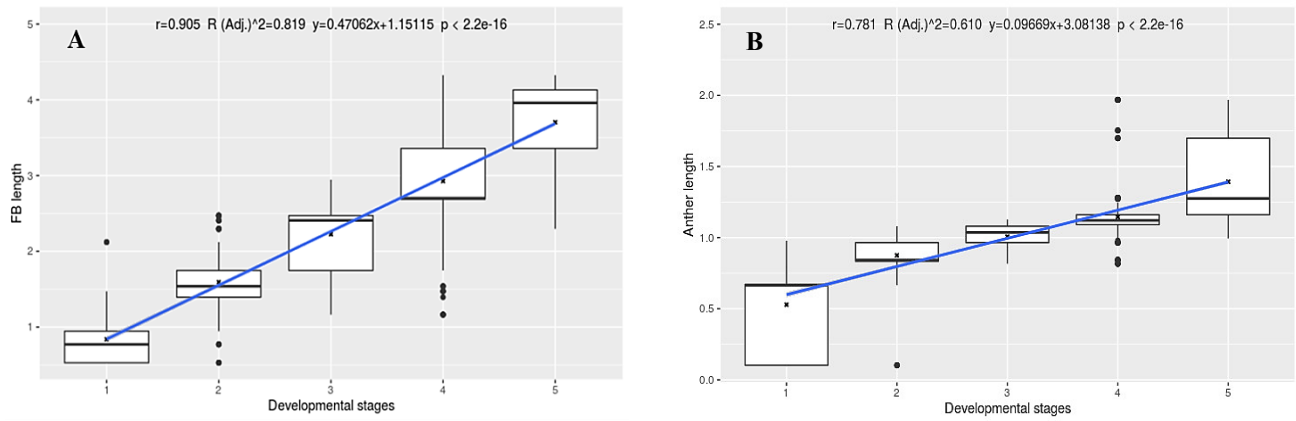

Fig. 5. Correlation coefficient (r) and Regression (R^2) between FB length (A), Anther length (B), and microspore developmental stages: 1=Meiocyte, 2=Tetrad, 3=Early uninucleate, 4=Late uninucleate, 5=Binucleate.

References

Adhikari, P.B. and W.H. Kang. 2017. Association of floral bud and anther size with microspore developmental stage in campari tomato. *Hort. Sci. & Technol.,* 35(5): 608-617.

Barroso, P.A, M.M. Rego, E.R. Rego and W. Soares. 2015. Embryogenesis in the anthers of different ornamental pepper (*Capsicum annuum* L.) genotypes. *Genet. & Mol. Res.,* 14(4): 13349-13363.

Blackmore, S., A.H. Wortley, J.J. Skvarla and J.R. Rowley. 2007. Pollen wall development in flowering plants. *New Phytol.,* 174(3): 483-498.

Carpenter, S.G. and G. Cottam. 1981. Growth and reproduction of American ginseng (*Panax quinquefolius*) in Wisconsin, U.S.A. *Can. J. Bot.*, 60(12): 2692-2696.

Diem, L.T., T.H. Phong, H.T. Tung, H.D. Khai, T.T.L. Anh, N.T.N. Mai, D.M. Cuong, V.Q. Luan, T. Que, H.T.N. Phuong, B.V.T. Vinh, and D.T. Nhut. 2022. Tetraploid induction through somatic embryogenesis in Panax vietnamensis Ha et Grushv. by colchicine treatment. *Scientia Horticulturae*, 303.

Ferrie, A.M.R. and K.L. Caswell. 2011. Isolated microspore culture techniques and recent progress for haploid and doubled haploid plant production. *Plant Cell Tiss. & Org. Cult.*, 104(3): 301-309.

Germana, M.A. 2011. Anther culture for haploid and doubled haploid production. *Plant Cell Tissue and Organ Culture,* 104: 283-300.

Han, N.R., S.U. Kim, H.Y. Park and H. Na. 2014. Microspore-derived Embryo Formation and Morphological Changes during the Isolated Microspore Culture of Radish (*Raphanus sativus* L.). *Kor. J. Hort. Sci. Technol.*, 32(3): 382-38.

Huong, N.T., K. Matsumoto and H. Watanabe. 1998. The antistress effect of majonoside R2, a major saponin component of Vietnamese ginseng: Neuronal mechanisms of action. *Methods Find Exp. Clin. Pharmacol.*, 20(1): 65-76.

Ibrahim, A.M., F.B. Kayat, M. Hussin, D. Susanto and M. Ariffulah. 2014. Determination of suitable microspore stage and callus induction from anthers of kenaf (*Hibiscus cannabinus* L.). *Sci. World J.,* 284-342.

Kim, Y.J., M.G. Jang, L. Zhu, J. Silva, X. Zhu, J. Sukweenadhi, W.S. Kwon, D.C. Yang and D. Zhang. 2015. Cytological characterization of anther development in *Panax ginseng* Meyer. *Protoplasma*, 253: 1111-1124.

Konoshima, T., M. Takasaki, H. Tokuda, H. Nishino, N.M. Duc, R. Kasai and K. Yámaki. 1998. Antitumor promoting activity of majonoside R2 from Vietnamese ginseng, *Panax vietnamensis* Ha et Grushv. *Biol. Pharm. Bull.,* 21(8): 834-838.

Lauxen, M., E. Kaltchuk-Santos, C. Hu, S.M. Callegari-Jacques and M.H. Bodanese-Zanettini. 2003. Association between floral bud size and developmental stage in soybean microspores. *Braz. Arch. Biol. & Technol.*, 46(4): 515-520.

Lewis, W.H. and V.E. Zenger. 1983. Breeding systems and fecundity in the American ginseng, *Panax quinquefolium* (Araliaceae). *Amer. J. Bot.,* 70(3): 466-468.

Lewis, W.H. and Zenger. 1982. Population dynamics of American ginseng, *Panax quinquefolium* (Araliaceae). *Amer. J. Bot.*, 69(9): 1483-1490.

Mai, T., T.N.H. Tran, T.T. Tran, T.L. Phan, D.G. Do, D.T. Bui, T.N.H. Nguyen, D.T. Pham, T.D, Le, D.M.H. Nguyen, V.K. Nguyen and H.H. Nguyen. 2014. Studies on induction and multiplication of somatic embryos of Ngoc Linh Ginseng (*Panax vietnamensis* Ha et Grushv.) in liquid medium. *J. Sci. & Devel.*, 12(7): 1085-1095.

Mangal, M. and A. Srivasatava. 2019. Exploitation of morphological features of bud and anther development for prediction of stages of microsporogenesis and microgametogenesis in pepper. *Ind. J. Exp. Biol.,* 57: 368-371.

Nguyen, M.L., T.H.T. Ta, T.N.B.T. Huyen and A.V. Voronina. 2019. Anther-derived callus formation in bitter melon (*Momordica charantia* L.) as conceived by microspore development stage and medium composition. *Sel'skokhozyaistvennaya Biol.,* 54: 140-148.

Nguyen, T.H. and T.T. Phuong. 2019. Vietnamese Ginseng (*Panax vietnamensis* Ha and Grushv.): Phylogenetic, Phytochemical, and Pharmacological Profiles. *Pharm. Rev.*, 13(26): 59-62.

Nguyen, T.T., P.T. Nguyen, T.H.N. Vu, K.H. Nguyen, N.H. Chu, B.N. Pham, H.H. Chu and Q.T. Phi. 2018. Isolation and screening of endophytic bacteria from ngoc linh ginseng (*Panax vietnamensis* Ha *et* Grushv) for biosynthesis B-glucosidase. *Acad. J. Biol.,* 40(2): 153-161.

Nham, N.T., P.V. De, T.C. Luan, N.M. Duc, S. Shibata, O. Tanaka and R. Kasai. 1995. Pharmacognostical and chemical studies on Vietnamese ginseng, Panax vietnamensis Ha et Grushv. (Araliaceae). *J. Jpn. Bot.*, 70(1): 1-10.

Parra-Vega, V., B. Gonzalez-Garcia and J.M. Sequi-Simarro. 2013. Morphological markers to correlate bud and anther development with microsporogenesis and microgametogenesis in pepper (*Capsicum annuum* L.). *Acta Physiol. Plant,* 35: 627-633.

Reunova, G.D., A.A. Reunov, Y.N. Aleksandrova, T.I. Muzarok and Y.N. Zhuravlev. 2007. Pollen Heteromorphism in *Panax ginseng* CA Meyer (Araliaceae) Anthers. *Dokl. Biol. Sci.*, 412: 76-78.

Sato, S., N. Kato, S. Iwai and M. Hagimori. 2002. Effect of low temperature pretreatment of buds or inflorescence on isolated microspore culture in *Brassica rapa* (syn. *B. campestris*). *Breed. Sci.,* 52: 23-26.

Schlessman, M.A. 1985. Floral biology of American ginseng (*Panax quinquefolius*). *Bull. Torrey Bot. Club,* 112(2): 129-133.

Schluter, C. and Z.K. Punja. 2000. Floral biology and seed production in cultivated North American Ginseng (*Panax quinquefolius*). *J. Amer. Soc. Hort. Sci.*, 125(5): 567-575.

Sumarmi, S., B.S. Daryono, D.P. Rachmawati and A. Indrianto. 2014. Determination of soybean (*Glycine max* L. [Merrill]) microspores development stage based on the length of flower buds. *J. Biol. Res.*, 20: 6-11.

Tran, Q.L., I.K. Adnyana, Y. Tezuka, Y. Harimaya, I. Saiki, Y. Kurashige, Q.K. Tran and S. Kadota. 2002. Hepatoprotective effect of majonoside R2, the major saponin from Vietnamese ginseng (*Panax vietnamensis*). *Planta Med.,* 68(5): 402-406.

Vu, T.H., Q.L. Vu, P.H. Nguyen, B.N. Nguyen, V.T.V. Bui, X.D. Thai and T.N. Duong. 2014. Direct somatic embryogenesis from leaf, petiole, and rhizome explant of *Panax vietnamensi*s Ha et Grushv. *J. Biol.*, 36(1): 277-282.

Wen, J. and J.W. Nowicke. 1999. Pollen ultrastructure of *Panax* (The ginseng genus, Araliaceae), an Eastern Asian and Eastern North American disjunct genus. *Amer. J. Bot.*, 86(11): 1624-1636.

Wilson, Z.A. and D.B. Zhang. 2009. From Arabidopsis to rice: Pathways in pollen development. *J. Exp. Bot.*, 60(5): 1479-1492.

Yobimoto, K., K. Matsumoto, N.T.T. Huong, R. Kasai. K. Yamasaki and H. Watanabe. 2000. Suppressive effects of vietnamese ginseng saponin and its major component majonoside-R2 on psychological stress-induced enhancement of lipid peroxidation in the mouse brain. *Pharm. Biochem. & Behavior*, 66(3): 661-665.

Zhu, S., H. Fushimi, S. Cai, H. Chen and K. Komatsu. 2003. A new variety of the genus *Panax* from Southern Yunnan, China and Its nucleotide sequences of 18S Ribosomal RNA gene and *mat*K gene. *J. Jpn. Bot*., 78: 86-94.

(Received for publication 15 September 2021)