Scientific Research Publishing

American Journal of Plant Sciences, 2017, 8, 2268-2281
http://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Florogenesis and Female Gametophyte Development in *Allium cepa* L. cv. Krishnapuram

Gujjula Vijaya Lakshmi[1], Mondam Muni Raja[1], Mude Lakshmipathi Naik[1], Sake Pradeep Kumar[2], Patan Shaik Sha Valli Khan[1*]

[1]Department of Botany, Yogi Vemana University, Kadapa, India
[2]Telecom Layout, Jakkur Layout, Jakkur (Po), Bangalore, India
Email: *pssvkhan@yahoo.com

How to cite this paper: Lakshmi, G.V., Raja, M.M., Naik, M.L., Kumar, S.P. and Khan, P.S.S.V. (2017) Florogenesis and Female Gametophyte Development in *Allium cepa* L. cv. Krishnapuram. *American Journal of Plant Sciences*, **8**, 2268-2281.
https://doi.org/10.4236/ajps.2017.89152

Received: July 8, 2017
Accepted: August 19, 2017
Published: August 22, 2017

Abstract

Florogenesis is one of the most complicated and interesting processes in the nature. This process involves developmental, physiological and molecular events leading to transformation from vegetative to reproductive phase for optimal seed production and the continuation of species. The basic knowledge about flowering processes, male and female sexual systems support basic and applied research and breeding programs. Most of the onion varieties from India are short day varieties, more diverse than other exotic germplasm and useful as a source of new alleles for supporting breeding programs. The present investigation was focused to study for the first time florogenesis process by scanning electron study for the first time florogenesis process by scanning electron microscopy (SEM) and development of female gametophytes by light microscopy in order to acquire basic knowledge useful for optimizing *in vitro* process to produce gynogenic haploid to support and speed breeding program in short-day onion *Allium cepa* L. cv. Krishnapuram (KP) or Bangalore Rose. This study revealed that shoot primordium differentiated into inflorescence meristem in the month of December, while seeds were planted in the field in the September. The individual florets are preceded by a variying number of floral initials. The female gametophyte developed from chalazal side megaspore. The embryo sac development is a bisporic *Allium* type showing short-lived antipodals. The histological study suggests that the use of big or pre-anthesis flower buds with embryo sac for production of gynogenic haploids to support breeding program in onion cv. Krishnapuram (KP). However further studies are needed for confirmation of this observation.

Keywords

Allium, Bangalore Rose, Female Gametophyte, Florogenesis, K.P. Onion, SEM

1. Introduction

The genus *Allium* (family: *Liliaceae*) comprises about 500 species distributed in the Northern hemisphere. The Cepa (Mill) Prokh section of *Allium* consists of nine wild species such as *A. cepa* (commonly known as bulb onion) and *A. fistulosum* (bunching onion). Onion is a historic vegetable crop considered to be originated in Central Asia and has been grown all over the world. It is cooked to combine flavor and served fresh in salads in the preparations of gastronomy. Traditional breeding and genetic methods have been employed to enhance onion quality, yield and tolerance to environmental stresses. However onion breeding efforts are hampered due to its biennial life cycle, heterozygosity and higher inbreeding depression. There is an urgent need to speed and support onion breeding programs to meet the consumer demand in the context of population pressure and climatic change [1]. To face with these problems, onion breeding programs should be integrated with recent plant molecular, biotechnology and omic techniques [2].

Florogenesis is one of the most complicated and interesting process in the nature. This process involves various developmental, physiological, biochemical and molecular events leading to conversion from vegetative to reproductive phase for optimal seed production for the continuation of the species. The basic knowledge about flowering process and development of sexual systems support basic and applied research and speed conventional breeding programs [2]. The florogenesis process is conveniently classified into five successive steps: induction, initiation, differentiation (organogenesis), maturation and development of floral parts and anthesis [3]. Flowering of diverse taxa within the genus *Allium* is greatly varied as per their developmental biology, morphology, genetic control and interaction with the growing environment [4]. The different biomorphological groups respond variedly to inductive conditions and thus flower in different ways. The process of florogenesis has been studied in very few species of *Allium* such as ornamental alliums [5], shallot [6] and garlic [7].

Both flowering and bulbing processes are important traits in *A. cepa* influenced by vernalization and photoperiodic responses, respectively [8]. With regard to temperature requirement, onions require cool temperatures during juvenile phase and warmer temperatures during maturity. Onion varieties are generally classified as short-day and long day varieties on the basis of the photoperiod. Various reports have indicated that long day and short-day germplasm are genetically dissimilar. These two groups further subdivided on the basis of geographical origins [9]. Moreover, it is found that most of the onion varieties from India are short-day varieties, further varied than other exotic germplasm and useful as a source of new alleles for breeding purposes [10]. The differences in the length of the juvenile phase (physiological age), the response to photoperiod and temperature required for floral induction have been already reported with long-day and temperate onion varieties [11].

Gynogenic haploids have been extensively and routinely used to support and

speed onion breeding programs. Gynogenic haploids have been obtained through *in vitro* culture using explants such as non-pollinated ovules, ovaries or flower buds [12]. It was also found that the developmental stage of explants is one of the critical aspects influencing gynogenic haploid production. The female gametophyte at different developmental stages has been used for induction of gynogenic haploids. Of the various stages tested. The mature embryo sac stage is proved to be more suitable than other developmental stages [13]. The analysis of the structure and development of the onion inflorescence and individual flower has been already reported [14]. Only one study could be found that was centered on the florogenesis process in long-day variety of onion [15]. However, these authors neither describe nor report the florogenesis and female gametophyte development in short-day varieties of onion. The present investigation was focused to study florogenesis process by scanning electron microscopy (SEM) and female gametophyte development by light microscopy in order to get basic knowledge useful for optimizing *in vitro* protocols to produce gynogenic haploids for supporting breeding programs in short-day onion cv. Krishnapuram (KP) or Bangalore Rose.

2. Materials and Methods

2.1. Plant Material

Umbels of *A. cepa* L. cv. Krishnapuram were collected from the agricultural fields located in mandal of Mydukur, Kadapa District, Andhra Pradesh, India in between the months of December-March. The umbels were divided into 7 groups based on their sizes and development *i.e.* non floral, very small floral, small floral, medium floral, above medium floral, big floral, very big floral. In each umbel consists flower buds (florets) ranges between 200 - 600. The individual flower buds were collected from very big floral stage of umbel and separated into four groups on the basis of their size as small buds (0.8 - 1.0 mm), medium buds (2.3 - 3.0 mm), big buds (3.1 - 3.7 mm) and pre-anthesis buds (3.8 - 4.4 mm) in diameter. Umbels and individual flower buds were photographically recorded by using a digital still camera.

2.2. Scanning Electron Microscopy

For scanning electron (SEM) examinations, umbels were fixed in 2.5% glutaraldehyde and 2% para formaldehyde in 0.1 M phosphate buffer (pH 7.4) in the field, kept in refrigerator at 4˚C and transported to the SEM facility at Sophisticated Analytical Instrument Facility (SAIF) at All India Institute of Medical Sciences (AIIMS), New Delhi. Samples were washed in 0.05 M phosphate buffer and dehydrated through a graded acetone series to 100% acetone. All samples were then critical-point-dried using a Bal-Tec 030 critical point dryer, mounted onto aluminum stubs with double-sided tape, layered with gold palladium using an Emitech K550 sputter coater. Scanning electron microscope (SEM) micrographs were obtained with Zeiss SEM S-4700) operating at 20 KV.

2.3. Microtomy and Light Microscopy

To study megasporogenesis and megagametogenesis ovaries from flower buds at four different stages of development [small, medium, big and pre-anthesis] were analyzed. Flowers were fixed in Carnoy's fluid (ethanol: acetic acid, 3:1) for 24 h at room temperature and then stored in 70% ethanol. Longitudinal serial (LS) sections were prepared according to standard techniques of wax embedding [16]. Embedded ovules were sectioned by rotary microtome for semi-thin sections. The staining of sections was carried out with safranin and toluidine-blue-O. The microscopic studies were examined under Carl-Zeiss (Germany) microscope. Micrographs were taken with CCD camera (Prog Res C3 Jenoptik).

3. Results and Discussion

The crop of *A. cepa* cv. Krishnapuram cultivated in the Southern part of India in two seasons known as *Kharif* (May-November) and *Rabi* (September-April). The growth cycle of this onion variety starts with seed germination, persists a survival period of vegetative growth and ends in the generative period. The present experiment was conducted from the field grown crop cultivated in the *Rabi* season (**Figure 1(a)**). The black rounded seeds were sowed in the month of September. The seeds were germinated after two weeks with the emergence of the

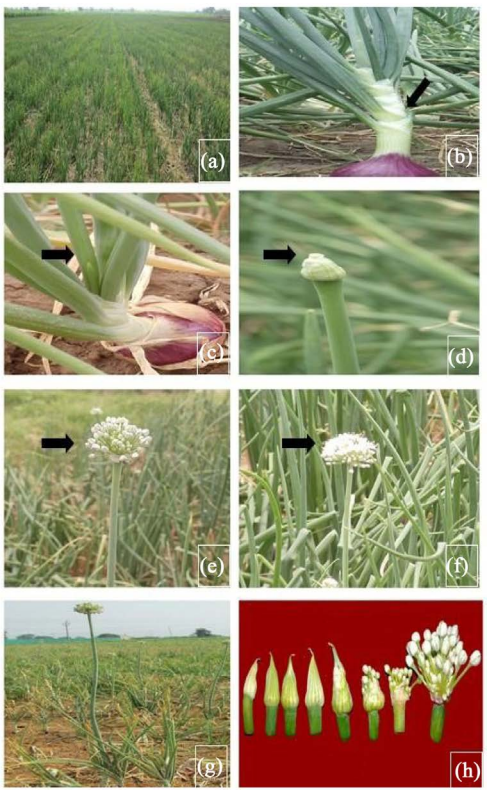

Figure 1. Morphology and floral development. (a) A field of onion crop; (b) Arrangement of leaves; (c) Initiation of floral stalk (scape initiation); (d) Umbel at small stage; (e) Umbel at medium stage (Developed); (f) A umbel with blooming; (g) Total plant; (h) Developmental stages of an umbel.

cotyledon, which pushes the embryonic rootlet downwards. One to two days afterward, the upper part of the cotyledon, grows an inverted U shape loop which is move forwarded upwards through the soil surface by elongation of two sides of cotyledon. In generally, onion crop is propagated by seeds, bulbs or sets. When grown from seed, the plants reported to go through vegetative and flowering stages of similar general appearance. The principle stages related to the growth of onion and other edible alliums from seed already reported by [17]. The earliest stages of development from seed are distinguished using the term loop, crook and first and second leaf stages. Where as in bulb propagated crops like garlic, shallot and rakkyo, the first leaf to report from the bulb penetrate to the soil surface is bladeless [18].

During vegetative growth, the stem is flattened to form a disc at the base of plant. At the top center of stem disc in the shoot apex, from which leaves are initiated oppositely and alternately (**Figure 1(b)**). Each leaf consists of blade and sheath. The sheath develops to completely encircle the growing apex. At the junction of the blade and sheath there is an opening or pore when the tip of the blade of the next younger most leaf can be seen. The blade of younger leaf was elongated and emerging through this pore. The apical meristem of vegetative shoot is flat and leaf primordial develops from the periphery towards centre. As new leaves are initiated and expand near the shoot apex, older sheath bases get pushed further away from the apex by continuing lateral expansion of the disc-like stem. The vegetative growth of stem remains till the end of the November. Thereafter, plant enters into the reproductive stage at 10 - 14 leaves stage. The onion Krishnapuram variety remained in vegetative stage for a period of 2 - 3 months *i.e.* from mid September to end of the November.

When grown from seed, all *Allium* plants require to reach a certain physiological age (for critical mass) prior being capable of florogenesis and blooming. The length of the juvenile phase is varied according to the species, it ranges from a few months in bulb onion [11], chives [19], Japanese bunching onion [20], leek [21] and shallot [11] to 5 - 6 years in *A. giganteum* and *A. karataviense* [22]. The duration of juvenile phase depends on the genotype as well as the growth environment. Both factors control the amount of stored reserves necessary for successful blooming. In addition to the available reserves, the ability to form flower also depends on the size of the apical meristem [3]. Further the transition from vegetative to the reproductive stage normally happens in the first or second growing season after the formation of 10 - 14 leaves in seed propagated alliums [11]. Under inductive conditions, floral initiation in shallot [6] and leek [21] is already possible after development of the first 6 and 7 true leaves (including leaf primordia), respectively.

The shoot apical meristem swells to form a dome shaped reproductive meristem in the month of December (**Figure 1(c)** and **Figure 1(d)**). SEM photograph **Figure 2(a)** shows non floral part of KP onion. Afterwards vegetative leaf initiation from meristem ceases. The juvenile *Allium* plants display monopodial growth habit and they become sympodial after the formation of the first genera-

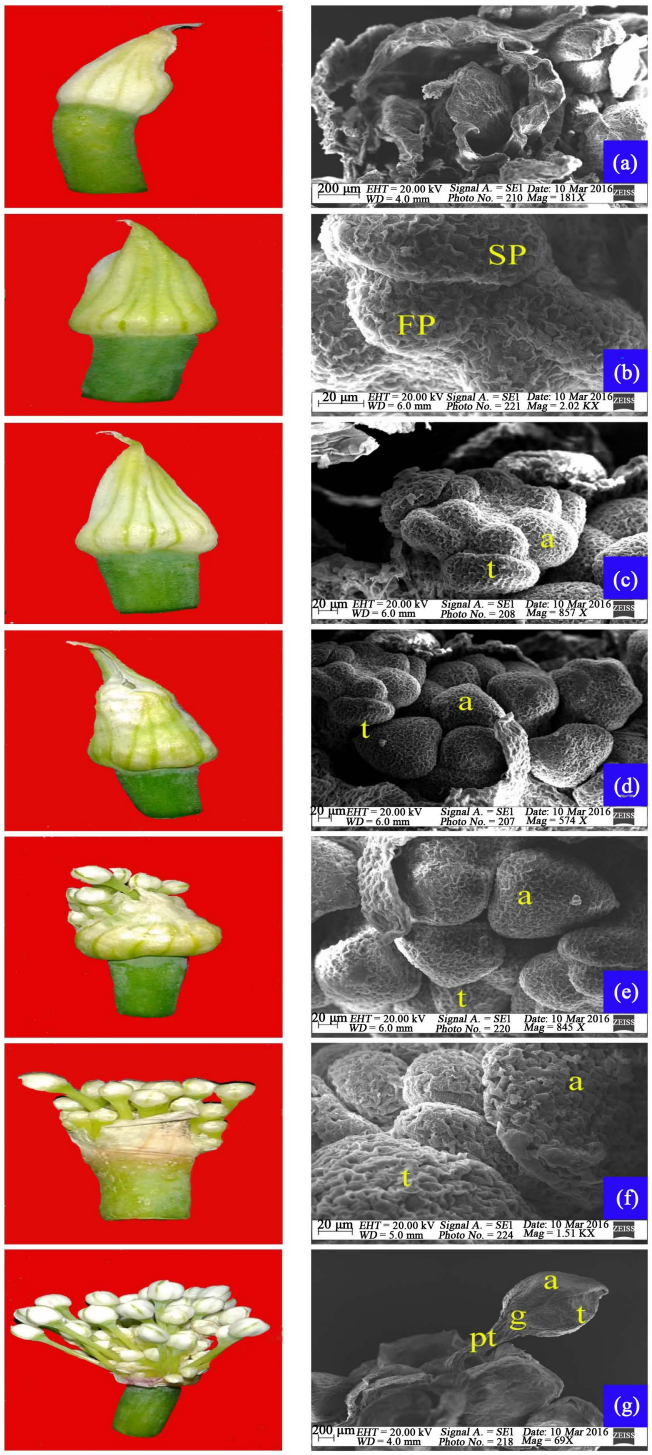

Figure 2. Scanning electron photomicrographs of floral development. (a) Vegetative apex, where the apical meristem ready to differentiate as reproductive meristem; (b) Initiation of flower primordial (FP), reproductive meristem was enveloped by the leaf like and papery spathe (SP); (c) Flower formation begins with subdivision of the apical meristem into four to several centers, development of complex monochasium cyme (bostryx); (d) and (e) Differentiate individual flower and floral parts like three tepals (t) and anthers (a) (g); (f) and (g) Finally developed inflorescence of *A. cepa* final stage og flower differentiation gynoecium segments (g) to form in the center of flower, and the anthers (a).

tive meristem. The reproductive meristem develops into an umbel type inflorescence that blooms by the mid February (**Figure 1(e)** and **Figure 1(f)**). After blooming, plant starts senescence and also complete bulb formation in the field (**Figure 1(g)**). The developmental process of umbel studied in seven stages as shown in **Figure 1(h)** by Scanning Electron Microscopy (SEM) based on the size, differentiation and development of flower buds (**Figure 2(a)**). The reproductive meristem was enveloped by the leaf like and papery spathe. The spathe consists of several membranous bracts (**Figure 2(b)**), which arises as close to uniform ring and elongates quickly to form more than 30 cm long inflorescence stalk known as scape. The scape is commonly hollow when mature, round in cross section and somewhat swollen at the middle or near the base. Similar observations were already reported in *Allium* sp. [1]. The proper elongation of scape elongation is essential for a successful bloom. This process strongly influenced by the storage and growth temperatures, as well as the photoperiod [23]. In garlic when the floral stalk attains 15 cm in length, the pedicles elongate and the inflorescence becomes spherical [11].

Flower formation begins with subdivision of the apical meristem into four to several centers. Each center gives rise to a numerous group of flowers clusters known as cymes. Each cluster has a spiral order of flower differentiation and development, which can be considered as a complex monochasium cyme (bostryx) (**Figure 2(c)**). New flower primordial are still being formed with in each cyme when the flower parts in the oldest flower primordial are already differentiated in the 1st week of January month [14] made an analysis of the structure and development of the onion inflorescence and of the individual flower [24] also reported an umbel-like flower cluster differentiation whose branches (cymes) come up from a common meristem. The umbel consists of 400 - 600 flowers in KP onion. The number of flowers per umbel differed within and between species and is greatly affected by environment, age and the position within the plant [25]. In KP onion, floral differentiation and organogenesis occur simultaneously with both scape elongation and vegetative growth and development as reported in other varieties of onion [7].

Individual flower buds or florets were differentiated over the surface of the reproductive meristem (**Figure 2(d)**). The five whorls of floral organs such as outer perianth, inner perianth, outer stamens, inner stamens and carpels were differentiated within the floret. The order of differentiation is distinct, but the exact sequence of development was not studied. The flower meristem develops initially as a slight projection, then turns into globose. The outer perianth whorl and the outer stamen whorl are the first to be formed. Inner whorls of perianth and stamens are formed after the outer (**Figure 2(e)**). Outer perianth and their associated stamens occur in a clockwise succession, whereas inner perianth arises together with their subtended stamens but in a counter clockwise direction (**Figure 2(f)**). The carpels are differentiated as three protruding areas when the outer perianth segments overarch the anthers. These three areas further develop

within the three inner stamens and alternate with them. These areas grow upward towards the center and meet at their edges. The apical growth of the three carpels results in the formation of a style. The trilocular ovary and gynoecium segments were developed in the centre of the flower (**Figure 2(g)**). Finally developed inflorescence of *A. cepa* final stage of flower differentiation gynoecium segments (g) to form in the center of flower, and the anthers (a) (**Figure 2(h)**). This flower opening was observed from oldest to youngest flowers within each cyme and over the whole surface of the umbel as reported earlier by [11]. The flower colour and the pattern of opening of individual flowers within the umbel vary with species. In shallot floral morphology is very similar to that of bulb onion, a clear direction of primordia differentiation in individual flowers has not been observed [6]. In garlic when compared with in onion and shallot, each perianth lobe and the subtended stamen arise at the same time from a single primordium [7].

3.1. Female Gametophyte

In *A. cepa* Krishnapuram onion has a trilocular ovary, and syncarpous gynoecium, which have superior ovary with two ovules on axile placentattion. Style is short and filiform and the stigma is minute. Based on the four different developmental stages of flower buds, the process of megasporogenesis and megagametogenesis was studied by using ovules (**Table 1**).

3.2. Megasporogenesis

The ovule consists of three basic structures such as a megasporangium, two integuments (inner and outer), and a funiculus, that are connected to the ovary wall. Early in the development of the pistil, ovular primordium and ovary wall differentiate simultaneously. The ovular primordium is composed of a group of meristematic cells located in the center (**Figure 3(a)**). Soon as archesporial cell, a large cell with dense cytoplasm is differentiated immediately below the epidermis. The periclinal division of archesporial cell leads to the formation of primary parietal cell and primary sporogenous cell. There after a megasporocyte or megaspore mother cell (MMC) formed from the primary sporogenous cell. The MMC can be distinguished among the other nucellus cells at a very early stage , even before the integuments begin to form at its base. The parietal cell divides periclinally 1 - 2 times to form 1 - 3 layers of nucellarcells, the ovule therefore is crassinucellate (**Figure 3(b)**). After differentiation, the inner integument is positioned at the nucellus, while the outer integument is located above the inner integument which contains two cell layers. At the top of the outer integument the micropyle pore is found. The funiculus is combined with one side of the ovule. This is observable as a rib (chalazal region) and situated at the opposite end of the micropyle.

The MMC go through a meiotic division and results in the formation of two unequal cells, a large chalazal and a small micropylar. The first meiotic division

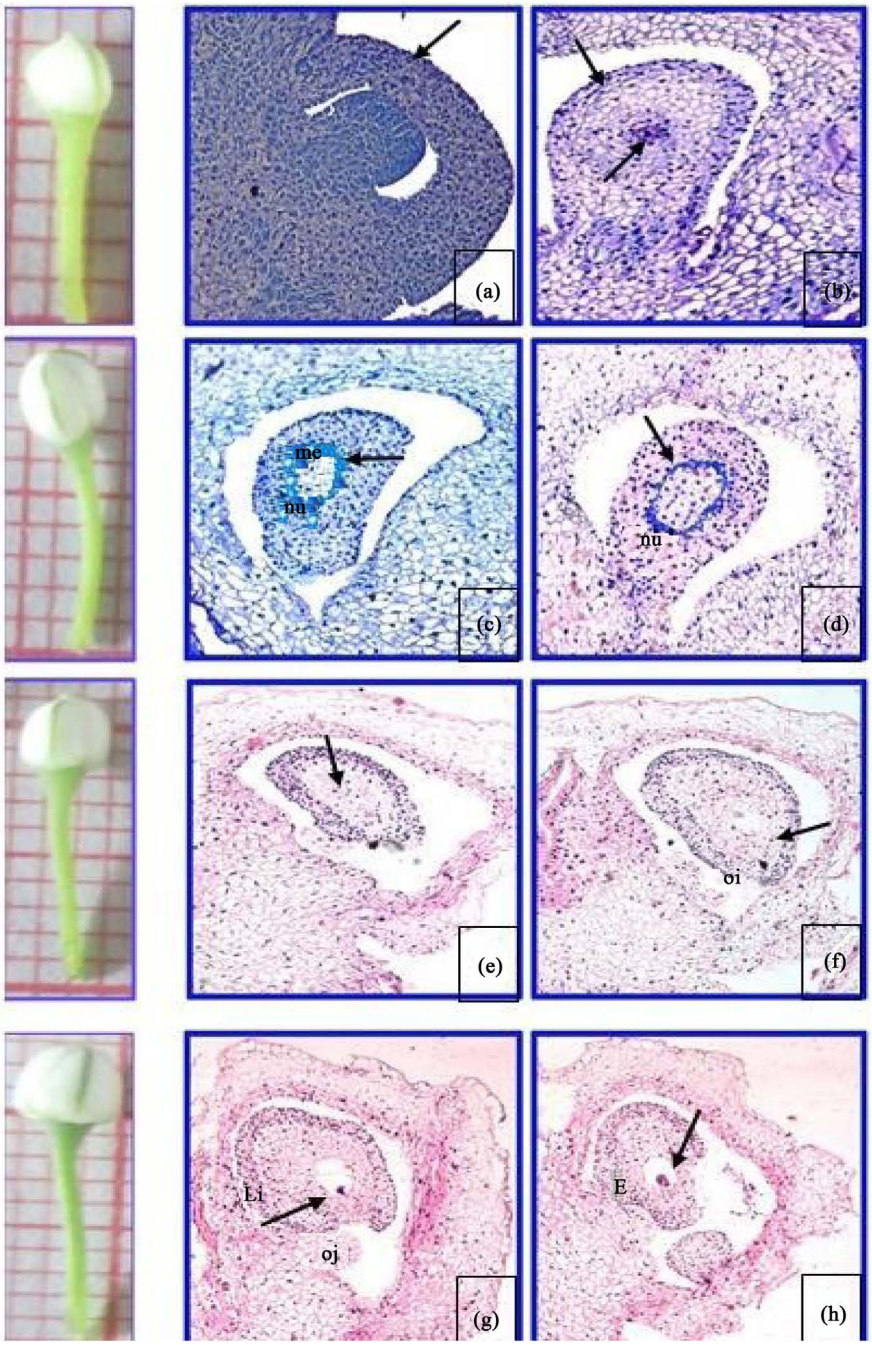

Figure 3. L.S. of ovaries and ovules at different stages of female gametophyte development. (a) Archesporial cell stage, initiation of integuments in ovule primordium (ovi); (b) Primary parietal cell and sporogenous cells, division of parietal cells and the formation of 1-3 layered nucellus cells. Megasporocytedeveloped; (c) Megasporocyte which slightly surrounded and begins to form a callose layer; (d) Megaspore mother cells divided and fully developed and surrounded by a thickened calloselayer; (e) Two-nuclei stage of development, one at micropylar and another situated at chalazal end; (f) At first mitotic division formation of four nuclei stage of embryo sac development; (g) At second mitotic division eight nuclei formed four at the end of micropylar and four at chalazal; (h) Early organized embryo sac showing three antipodal cells, one egg apparatus cell with two synergids and two centrally located polar nuclei (seven celled-eight nuclei).

Table 1. Morphological indications and respective lengths of floral buds in *A. cepa* L. Krishnapuram at various stages of development.

S. no	Flower Development Stage	Bud length (mm)	Morphological indications	Figure Number
1.	Small	0.8 - 1.0	Archesporial cell stage, initiation of integuments in ovule primordium (ovi). Primary parietal cell and sporogenous cells, division of parietal cells and the formation of 1 - 3 layered nucellus cells. Megasporocyte developed.	**Figure 3(a)** & **Figure 3(b)**
2.	Medium	2.3 - 3.0	Megasporocyte which slightly surrounded and begins to form a callose layer Megaspore mother cells divided and fully developed and surrounded by a thickened callose layer.	**Figure 3(c)** & **Figure 3(d)**
3.	Big	3.1 - 3.7	Two-nuclei stage of development, one at micropylar and another situated at chalazal end. At first mitotic division formation of four nuclei stage of embryo sac development	**Figure 3(e)** & **Figure 3(f)**
4.	Pre-anthesis	3.8 - 4.4	At second mitotic division eight nuclei formed four at the end of micropylar and four at chalazal. Early organized embryo sac showing three antipodal cells, one egg apparatus cell with two synergids and two centrally located polar nuclei (seven celled–ight nuclei).	**Figure 3(g)** & **Figure 3(h)**

is also accompanied by cytokinesis. After some time the small micropylar cell degenerates and only the chalazal cell The second meiotic division of the chalazal cell gives two functional megaspores. The second meiotic cell division does not associate with cell wall formation, begins to deposit of callose around the megasporocyte (**Figure 3(c)**). Cell wall formation after meiosis I of megasporogenesis seems to be of universal occurrence in all plants [26]. The functional megaspore mother cells at the stage of development fully surrounded by callose (**Figure 3(d)**). In case of *A. consanguineum* a species growing wild in the Western Himalayas, the cell wall is not laid after the first meiosis [27]. The degeneration of the micropylar daughter cell in *A. cepa* is characteristic of many species of *Allium* and occurs in other genera as well. The origin of the embryo sac from the chalazal daughter cell has also been described for *A. fistulosum* [28], *A. porrum*, *A. victoriale*, *A. paniculatum*, *A. flavum*, *A. ursinum*, *A. edbdaneese*, *A. uniflorum*, *A. rotundum*, and *A. sphaerocephalum*, *A. carnatum*, *A. oleraceum*, *A. scorodoprasum*, *A. moly bulbiferum*, *A. paradoxum*, and *A. sativum* [29].

3.3. Megagametogenesis

Megagametogenesis process begins with increase in the size of the female gametophyte. The mitosis-I division of two megaspores nuclei produces four nuclei,

two situated at the chalazal end and the other two at the micropylar pole. These nuclei were alienated by a large vacuole in the centre of the embryo sac without cytokinesis (**Figure 3(e)**). Later on these four nuclei go through mitosis-II to gives rise eight nuclei. The four nuclei are positioned at the chalazal end and the four at the micropylar end. The mitotic division I & II occur in a highly coordinate manner at both ends of the embryo sac (**Figure 3(f)**). Two successive mitotic divisions and absence of cytokinesis in long day varieties of onion. In general, the cellularizedbisporic embryo sac contains seven cells (eight nuclei): three antipodal cells situated at the chalazal end, two polar nuclei and three celled egg apparatus [14]. However, we did not observe the presence of antipodals in the embryo sac. Further observations revealed that the egg apparatus is lied in close contact with the micropyle (**Figure 3(g)** and **Figure 3(h)**). Several variations were reported in *Allium* type of embryo sac development, [30]. The absence of the antipodals may be due to their rapid degeneration or may be unnoticed as they concealed at the end of chalazal side [26]. In other varieties of *A. cepa*, the presence of antipodals has not been reported in all published papers [12]. The existence of short lived antipodal cells has also been reported in *Chichoriumintybus* [31] and *Agave tequilana* [32]. In addition to that, the close contact of the egg apparatus with the micropyle is highly similar to what was observed in the ovular apparatus of *Ornithogalum caudatum* [33]. Moreover, as observed in many other angiosperms, the egg cell possesses a highly dense nucleus placed towards the chalazal extreme of the embryo sac [34].

4. Conclusion

Seeds of *A. cepa* L. cv. Krishnapuram onion used for bulb production in the field in two seasons *Rabi* and *Kharif* during, September-April and May to November, respectively. It was found that the primordium of the inflorescence axis differentiated in the month of December while seeds were planted in the field in September. The individual florets are preceded by a varying number of floral initials in the month of December. The female gametophyte was developed from chalazal side megaspore. The embryo sac development is a bisporic *Allium* type, showing short-lived antipodals. The observations reported here show basic information on florogenesis process and the female gametophyte development to support breeding programs. The gynogenic response is strongly depended on the developmental stage of female gametophyte. The present histological study correlated with the developmental stages according to the size of the flower buds (small, medium, big, pre-anthesis) (**Table 1**). Gynogenic development start only after a mature embryo sac has been organized. This observation might suggest the use of big or pre-anthesis flower buds with embryo sac for the production of gynogenic haploids to support breeding program in onion cv. Krishnapuram. However, further studies are needed for confirm this observation.

Acknowledgements

This work was supported by the financial assistance to GVL in the form of

INSPIRE Fellowship by the Department of Science and Technology, Government of India, New Delhi. The authors would like to thank SAIF-AIIMS, New Delhi for providing SEM facility for studying florogenesis process.

References

[1] Brewster, J.L. (2008) Onions and Other Vegetable *Allium*s. CABI, Oxford. https://doi.org/10.1079/9781845933999.0000

[2] Kosa, J., Callum, J., Dhatt, A. and Macknight, C. (2016) Enhancing Onion Breeding Using Molecular Tools. *Plant Breeding*, **135**, 9-20. https://doi.org/10.1111/pbr.12330

[3] Nard, M. and Hertogh, A.A. (1993) Bulb Growth and Development and Flowering. In: De Hertogh, A.A. and Le Nard, M., Eds., *The Physiology of Flower Bulbs*, Elsevier, Amsterdam, 29-44.

[4] Baldwin, S.R., Revanna, M., Pither-Joyee, M., Shaw, K., Wright, S., Thomson, L., Moya, R., Lee, R., Macknight, R. and Callum, J. (2014) Genetic Analyses of Bolting in Bulb Onion (*Allium cepa* L.). *Theoretical and Applied Genetics*, **127**, 535-547. https://doi.org/10.1007/s00122-013-2232-4

[5] Kamenetsky, R. and Japarova, N. (1997) Relationship between Annual Cycle and Floral Development of Three *Allium* Species from Subgenus *Melanocrommyum*. *Journal of Arid Environments*, **35**, 473-485. https://doi.org/10.1006/jare.1996.0161

[6] Krontal, Y., Kamenetsky, R. and Rabinowitch, H.D. (1998) Lateral Development and Florigenesis of a Tropical Shallot—A Comparison with Bulb Onion. *International Journal of Plant Science*, **159**, 57-64. https://doi.org/10.1086/297521

[7] Kamenetsky, R. and Rabinowitch, H.D. (2001) Floral Development in Bolting Garlic. *Sexual Plant Reproduction*, **4**, 235-241. https://doi.org/10.1007/s004970000061

[8] Lee, R., Baldwin, S., Kenel, F, Callum, J. and Macknight, R. (2013) Flowering Locus T Genes Control Onion Bulb Formation and Flowering. *Nature Communications*, **4**, 2884. https://doi.org/10.1038/ncomms3884

[9] Khokar, K.M. (2008) Effect of Temperature and Photoperiod on the Incidence of Bulbing and Bolting in Seedlings of Onion Cultivars of Diverse Origin. *Journal of Horticultural Science and Biotechnology*, **83**, 488-496. https://doi.org/10.1080/14620316.2008.11512412

[10] Baldwin, S.M.P., Joyce, K., Wright, K., Chen, L. and Callum, J. (2012) Development of Robust Genomic Simple Sequence Repeat Markers for Estimation of Genetic Diversity within and among Bulb Onion (*Allium cepa* L.) Populations. *Molecular Breeding*, **30**, 1401-1411. https://doi.org/10.1007/s11032-012-9727-6

[11] Rabinowitch, H.D. (1990) Physiology of Flowering. In: Rabinowitch, H.D. and Brewster, J.L., Eds., *Onions and Allied Crops*, CRC Press, Boca Raton, Vol. 3, 113-134.

[12] Musial, K., Bohanec, B., Jakse, M. and Przywara, L. (2005) The Development of Onion (*Allium cepa* L.) Embryo Sacs *in Vitro* and Gynogenesis Induction in Relation to Flower Size. *In Vitro Cell Developmental Biology-Plant*, **41**, 446-452. https://doi.org/10.1079/IVP2005645

[13] Mishra, V.K. and Goswami, R. (2014) Haploid Production in Higher Plant. *International Journal of Chemical and Biological Sciences*, **1**, 25-45.

[14] Jones, H.A. and Emsweller, S.I. (1936) Development of the Flower and Macrogametophyte of *Allium cepa*. *Hilagardia*, **10**, 415-428. https://doi.org/10.3733/hilg.v10n11p415

[15] De Mason, A. (1990) Morphology and Anatomy of *Allium*. In: Rabinowitch, H.D. and Brewster, J.C., Eds., *Onions and Allied Crops*, CRC Press, Boca Raton.

[16] Johansen, D.A. (1940) Plant Microtechnique. McGraw-Hill, London.

[17] Rey, C., Stahl, J., Antonin, P. and Neury, G. (1974) Stadesreperes de l'oignon de semis. *Revue Suisse de Viticulture*, *Arboriculture et Horticulture*, **6**, 101-104.

[18] Mann, L.K. (1959) The *Allium* Inflorescence: Some Species of the Section Molium. *American Journal of Botany*, **46**, 730-739. https://doi.org/10.2307/2439129

[19] Poulsen, N. (1990) Chives *Allium schoenoprasum* L. In: Rabinowitch, H.D. and Brewster, J.L., Eds., *Onions and Allied Crops*, *III Biochemistry*, *Food Science*, *and Minor Crops*, CRC Press, Boca Raton, 231-250.

[20] Inden, H. and Asahira, T. (1990) Japanese Bunching Onion (*Allium fistulosum* L.). In: Brewster, J.L. and Rabinowitch, H.D., Eds., *Onions and Allied Crops*, *III*: *Biochemistry*, *Food Science*, *and Minor Crops*, CRC Press, Boca Raton, 159-178.

[21] Van der Meer, Q.P. and Hanelt, P. (1990) Leek (*Allium ampeloprasum*). In: Brewster, J.L. and Rabinowitch, H.D, Eds., *Onions and Allied Crops*, *I*: *Botany*, *Physiology and Genetics*, CRC Press, Boca Raton, 179-196.

[22] Hertogh, A.A. and Zimmer, K. (1993) *Allium*-Ornamental Species. In: De Hertogh, A.A. and Le Nard, M., Eds., *The Physiology of Flowering Bulbs*, Elsevier, Amsterdam, 187-200.

[23] Kamenetsky, R. and Rabinowitch (2002) Florogenesis. In: Rabinowitch, H.D. and Currah, L., Eds., *Allium Crop Science*: *Recent Advances*, CAB International, Wallingford, 31-58.

[24] Kamenetsky, R. (1997) Inflorescence of *Allium* Species (Subgenus Melanocrommyum): Structure and Development. *Acta Horticulturae*, **430**, 141-146. https://doi.org/10.17660/ActaHortic.1997.430.19

[25] Ali, M., Dowker, B.D., Currah, L. and Mumford, P.M. (1984) Floral Biology and Pollen Viability of Parental Lines of Onion. *Annals of Applied Biology*, **104**, 167-174. https://doi.org/10.1111/j.1744-7348.1984.tb05599.x

[26] Maheshwari, P. (1950) An Introduction to the Embryology of Angiosperms. McGraw-Hill, New York. https://doi.org/10.5962/bhl.title.5681

[27] Gohil, R.N. and Kaul (1981) Studies on Male and Female Meiosis in Indian *Allium*. *Chromosoma*, **82**, 735-739. https://doi.org/10.1007/BF00285778

[28] Strasburger, E. (1879) Die Angiospermen, D. and die Gymnospermen. Gustav Fischer, Jena. https://doi.org/10.5962/bhl.title.20324

[29] Weber, E. (1929) Entwicklungsgeschichtliche Untersuchungenuber die Gattung *Allium*. *Botanisches Archives*, **25**, 1-44.

[30] Johri, B.M., Ambegaokar, K.B. and Srivastava, P.S. (1992) Comparative Embryology of Angiosperms. Springer-Verlag, Berlin, 863-871. https://doi.org/10.1007/978-3-642-76395-3

[31] Chehregani, A., Mohsenzade, F. and Ghanad, M. (2011) Male and Female Gametophyte Development in *Cichoriumintybus*. *International Journal of Agricultural Biology*, **13**, 603-606.

[32] Gutierrez-Mora, A., Gonzalez-Gutierrez, A.G., Rodriguez-Garay, B., Ascencio-Cabral, A. and Li-Wei, L. (2012) Plant Somatic Embryogenesis: Some Useful Considerations. In: Ki, S., Ed., *Embryogen*esis, InTech, Rijeka, 229-241.

[33] Tilton, V.R. (1978) A Developmental and Histochemical Study of the Female Reproductive System in *Ornithogalum caudatum* Ait. Using and Electron Microscopy.

PhD Dissertation, Iowa State University, Ames.

[34] Tian, H.Q., Yuan, T. and Russell, S.D. (2005) Relationship between Double Fertilization and the Cell Cycle in Male and Female Gametes of Tobacco. *Sexual Plant Reproduction*, **17**, 243-252. https://doi.org/10.1007/s00497-004-0233-9