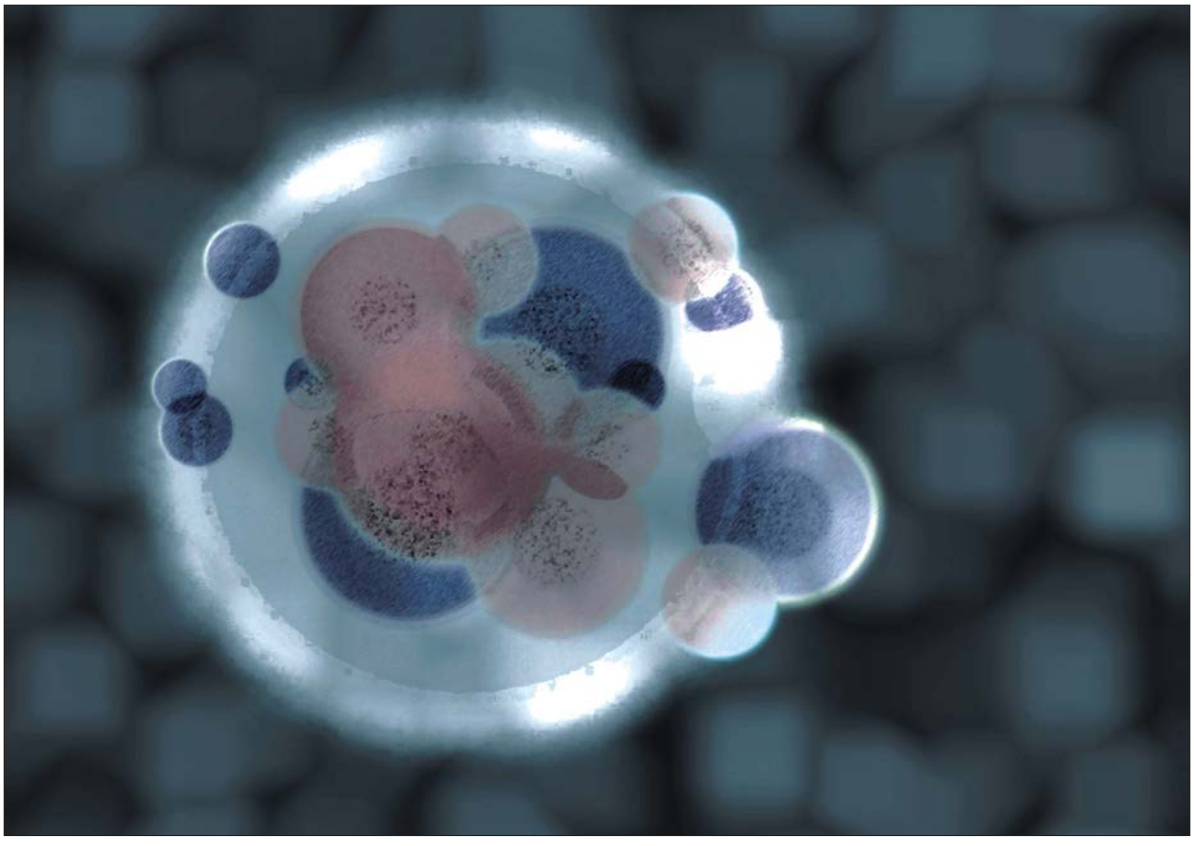

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A Developmental Study of the Inflorescence and the Flower of Rapeseed (*Brassica napus*. cv. Hyola) Usimg Epi-Illumination Light Microscopy

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Abstract: Ontogeny and the developmental pattern of flower organs of *Brassica napus* (cv. Hyola) were investigated using epi-illumination microscopy and image analyzing. Similar results as compared with Scanning Electron Microscopy (SEM) were obtained with this simplified and amenable procedure. Using this microscopy technique we have demonstrated that after the transition of the vegetative apex into the reproductive apex in the 3-leaved seedlings, flower primordia initiation takes place in a helical phyllotactic pattern and the initiation of flower organs pursues the following steps; sepals, stamens, gynoecium and petals. In the formation of four sepals, the abaxial sepal primordium initiated first and it was followed by the formation of the two others in the lateral position. The adaxial sepal primordium was initiated in the last step. Of the six stamens, the four long ones, were initiated first and they were positioned in an alternating order with the four sepals. The two short stamens were initiated in a basipetal manner and in the outside of the long stamens, opposite to the lateral sepals. The remainder part of the apex grows upward to produce an oval, hollow tube which will form the gynoecium. Petal primordia grow slowly and they were initiated after stamens and gynoecium initiation on either side of the two short stamens. The gynoecium at maturity was characterized with a papillated stigma and an elongated style. The ovary was long and divided by a septum in the internal side of the ovary. Sepals, style and the abaxial zone of anthers showed stomatas but they were absent in the petals.

Key words: Development, epi-illumination, floral ontogeny, cruciferera, rapeseed

INTRODUCTION

The recent position and importance of rapeseed (*Brassica napus* L.) in oil seed production has been accompanied by focusing research programs on the seed yield and quality improvement. Indeed, rapeseed oil is the third most important edible oil after soybean and palm in the world (Robbelen *et al*., 1989). In this respects ontogeny and the study of developmental pattern of floral organs and seed production seems necessary and informative. The transition of the vegetative meristem into the reproductive apex and the initiation of floral organs in several members of cruciferae have been studied in detail (Chakravarti, 1953; Sadik and Ozbum, 1967; Sattler, 1973; Orr, 1978.).

The use of Scanning Electron Microscope (SEM) has expanded and detailed our knowledge of apex transition and floral organs development (Turker, 1982, 1984, 1985; Rosenberg and Bonnett, 1983; Polowick and Sawhney, 1986; Smyth *et al*., 1990; Prenner, 2003). This technique provides a 3-dimentional image that makes it easier to study the apex development. As compared with SEM, similar results on organ initiation as compared with SEM may also be obtained using epi-illumination light microscopy (Sattler, 1968; Charlton *et al*., 1989) accompanied by the application of new computerized image analysis in a practical and simplified manner. In this study we have tried to show the efficiency of the epi-illumination light microscopy application and 3-dimentional image construction during the development of floral organs in *B. napus*.

MATERIALS AND METHODS

Brassica napus cv Hyola seeds (a spring variety) were obtained from the Oilseed improvement department of the Seed and Plant Improvement Institute (SPII, Karaj. IRAN). Seeds were planted in the fall of 2005 in a mixture of sand, loam and peat (1; 2 ;1) in 15 cm plastic pots. Pots were placed in a greenhouse with a 16d/8n photoperiod and the temperature was controlled at 18°C at night and 25°C during the day time. Sampling was started when the

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second leaf in the seedlings was appeared and it was continued with the appearance of the subsequent leaves until the visible appearance of the floral buds. All specimens were immediately fixed in FAA (formalin 5%, acetic acid 5% and 70% ethanol 90%) for 24 h at room temperature according to Posluszny *et al.* (1980). Then, the samples were transferred into 70% ethanol for 24 h. and subsequently into 96% ethanol at room temperature (the specimens may be conserved at 96% ethanol for a long period). The specimens were dissected under a SZX9 Olympus Stereomicroscope in 96% ethanol and stained with nigrosin to increase the contrast of the samples. The specimens were transferred into 96% ethanol for 3 days to eliminate the excess nigrosin. Samples were mounted on a sharp insect needle which was fixed in the center of Petri dishes in 96% ethanol, such that it was possible to adjust the orientation of specimens for suitable angle of microphotography. Olympus BX51 research epi-illumination microscope was used and digital images were prepared by TIFF format at the RGB mode (12 bits per each channel) and at the resolution of 1344×1024 pixels.

Digital images were transferred into the Photoshop 7 software (Adobe Company) and the image level, contrast and hue were adjusted. Complete images were finally flattened and saved as 300 dpi uncompressed file for hard copy print.

RESULTS AND DISCUSSION

Organ initiation during plantlet development: In our culture conditions, the microscopic study of the first specimens obtained from 2-leaved seedlings showed only a vegetative apex located among the leaf primordia before its conversion to the reproductive apex. The meristem dome was slightly convex (Fig. 1). The vegetative apex was clearly visible after the removal of the leaf primordium (Fig. 2).

Under our growth conditions the appearance of the third leaf was the beginning of the vegetative apex conversion into the reproductive apex (Fig. 3 and 4). At this stage the apical dome became wider similar to that described by Polowick and Sawhney (1986) and Orr (1978). In the 4-leaved specimens the initiation of the inflorescence with the appearance of the first floral primordium was demonstrated and the development of the floral buds in a phylotactic spiral pattern was observed (Fig. 5 and 6). This observation could be followed up in the subsequent specimens which were prepared from seedlings having 5 leaves and up (Fig. 7). In the specimens prepared from plantlets with 5 leaves inflorescence initiation was accomplished (Fig. 8). The spiral phylotactic pattern was observed in both the terminal and on the branch inflorescence. The inflorescence was initiated from the axils of each leaf in the branches (Fig. 9 and 10).

In the specimens prepared from 6-leaved seedlings, sepals were the first organ which was initiated in the flower buds on the inflorescence. The four sepal precursors were initiated as broad ridges at the edges of the floral meristem and continued to elongate. The abaxial sepal primordium was arisen first followed by the adaxial and the two laterals (Fig. 11). Different specimens from 6-leaved plantlets demonstrate well the sepals development and complete pattern formation (Fig. 11 and 12). The initiation of sepals was followed by the initiation of the stamens instead of the petals. This was demonstrated in the specimens prepared from 7-leaved plantlets after the removal of the sepals (Fig. 14). This pattern of initiation has been previously described in Crusifera and *B. napus* (Hayword, 1938; Polowick and Sawhney, 1986; Smyth *et al*., 1990). This developmental pattern is largely different from the normal acropetal pattern of floral development which was described by Sattler (1973). At this stage, the gynoecium primordium was clearly demonstrated in our study which was seen surrounded by the stamens (Fig. 13).

Four of the six stamens, which were long, were initiated in antepetalous positions and the two short stamens were initiated in antesepalous position (Fig. 14). These two stamens were initiated in outside and basipetal to the long stamens (Fig. 15). In the specimens fixed from 7-leaves plantlets all sepals were fully developed and curved inward to cover the apex (Fig. 13). Four of the six stamens were bulged out and the long stamens became distinct from the central part of the apex dome (Fig. 14 and 16). Specimens prepared from 8-leaved plantlets demonstrate well this pattern of development. This stamen pattern of development did not follow the centripetal pattern of stamen initiation described by Sattler (1973), but it was classified on the centrifugal pattern of stamen development (Cronquist, 1968).

In the specimens prepared from 8-leaved plantlets the primary steps of gynoecium development were accomplished (Fig. 15) and the four petal primordia were arisen next on either side of the two short stamens (Fig. 15 and 16). These primordia were barely visible until all six stamens were developed enough and begun to enlarge (Fig. 16).

In the fixed specimens from 8-leaved plantlets, in order to observe and study the internal floral organs development it was necessary to manually remove the sepals. In these specimens from 8 and 9 leaved plantlets the comparison of the petal size with stamen size showed

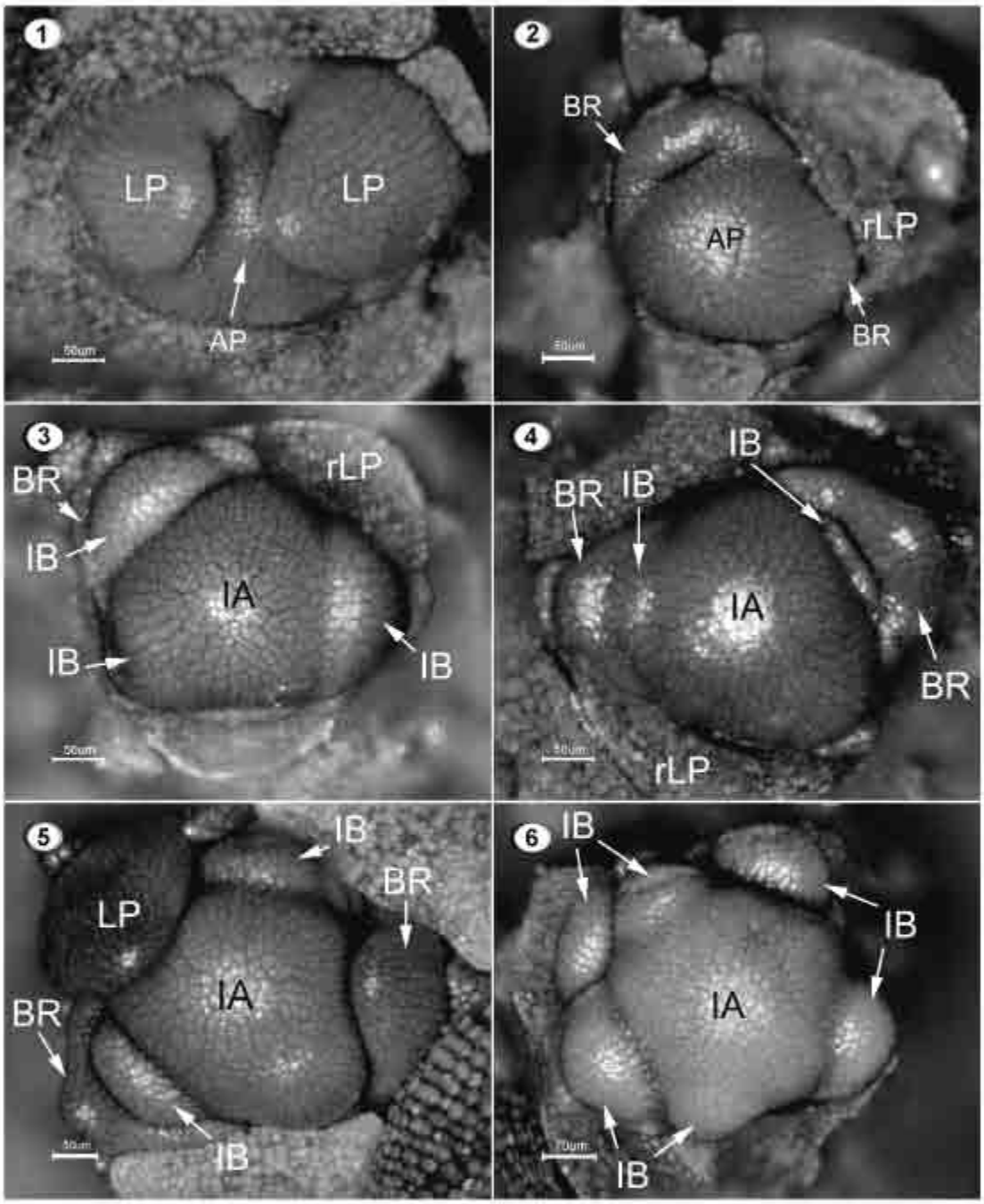

Fig. 1-6: 1. A vegetative apex with two young leaf primordia. 2. A dome shape apex, the beginning of meristem transition. 3. A young inflorescence apex with primitive bud primordia. 4-5. Young developing inflorescence. 6. A young developing inflorescence showing the helical pattern of flower bud initiation. LP = Leaf Primordia; Br = Bractea; rLP = Removed Leaf Primordial; IA = Inflorescence Apex; IB = Inflorescence Bud

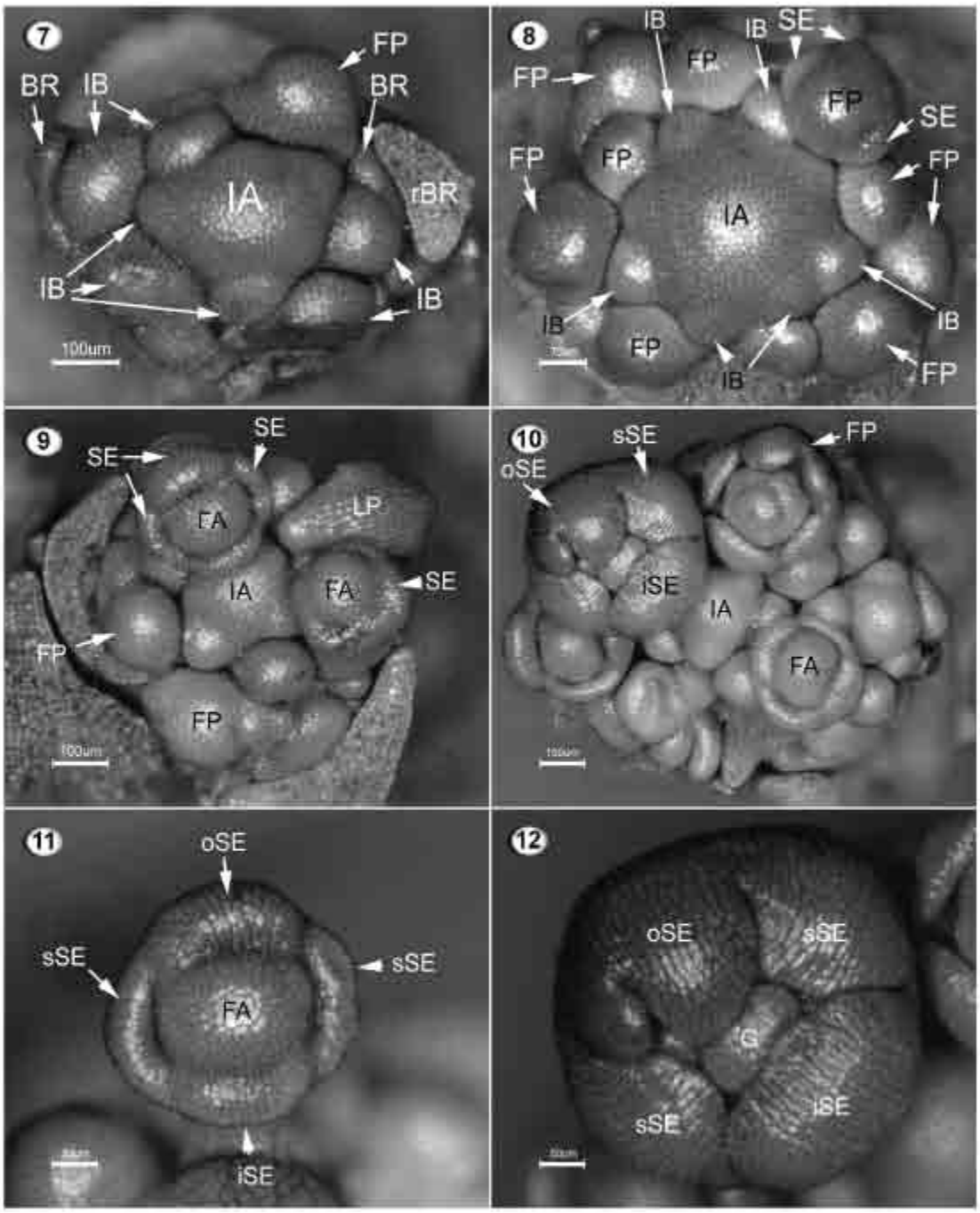

Fig. 7-12: 7-8. A developing inflorescence showing floral initiation. 9. Initiation of young flower bud organs. 10. Developed stage of flower bud organs initiation. 11. A young flower bud. 12. A flower bud with a well developed sepals and gynoecium initiation. FP = Flower Primordia, FA = Flower Apex; oSE = Outer (abaxial) Sepal; iSE = Inner(adaxial) Sepal; sSE = Side Sepal; G = Gynoecium

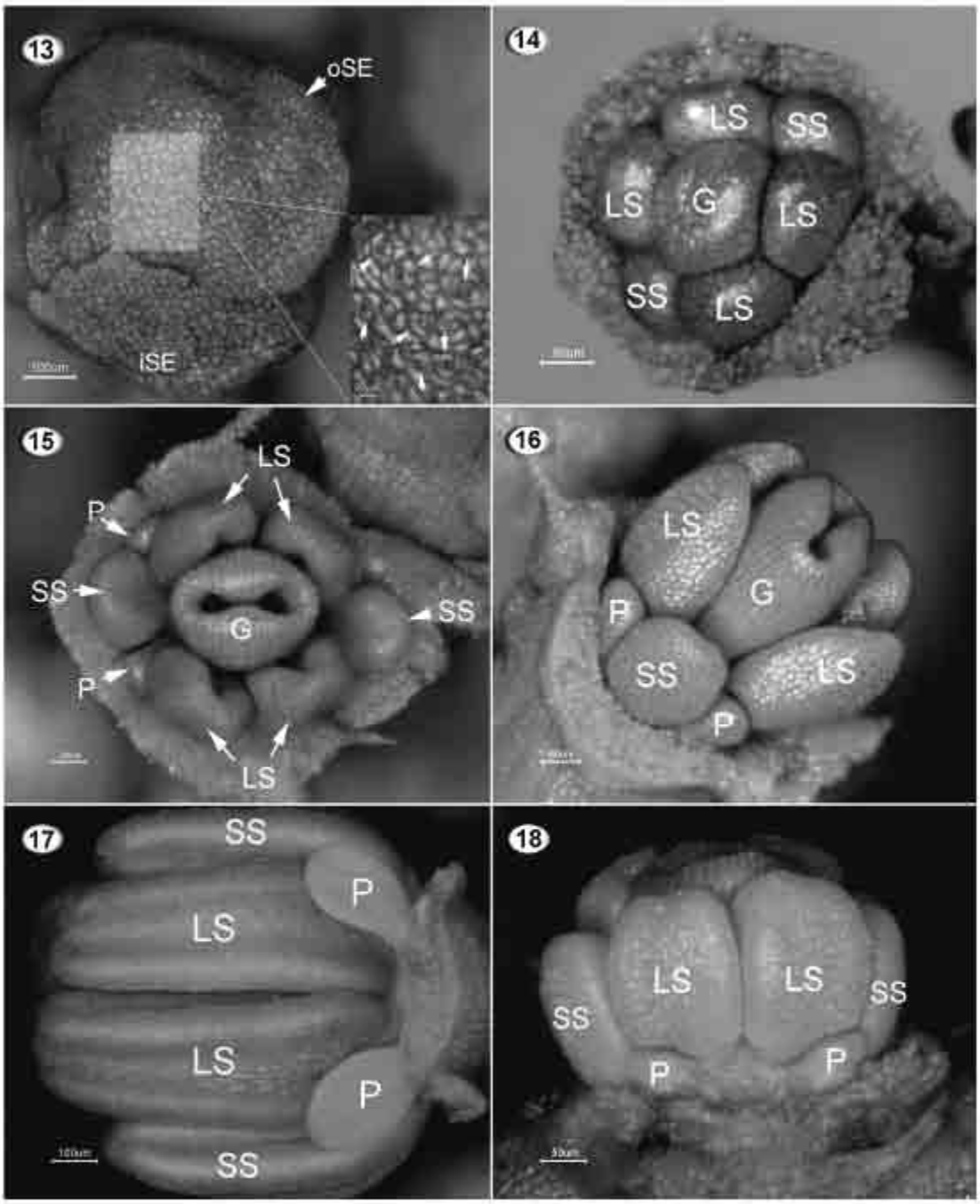

Fig. 13-18: 13. Young flower bud with developed sepals enclosing all other flower organs, arrows indicate the stomata distribution. 14. Young flower bud with removed sepals showing the position of the stamens and gynoecium, note that the petal initiation was not yet detectable. 15-16. Developing stage of flower bud, same growth rate for stamens and gynoecium. Petals are initiated and located on either side of the short stamens. 17-18. Developing stage of flower bud showing the relative arrangement of the petals and stamens. LS = Long Stamen; SS = Short Stamen; G = Gynoecium; P = Petal

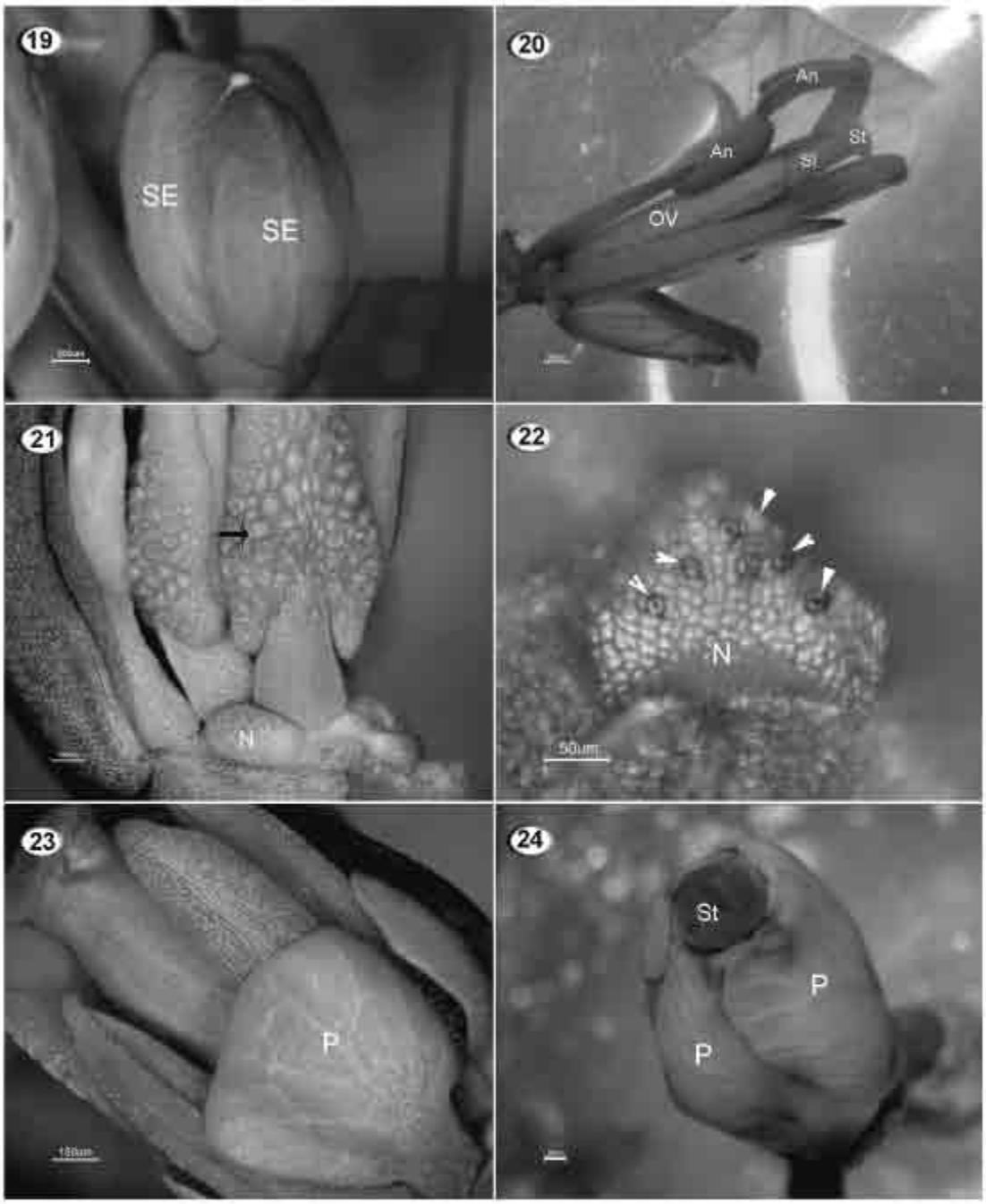

Fig. 19-24: 19. Sepals position in a developing flower bud enclosing the all other flower organs. 20. A mature flower after removing sepals and petals, showing mature stamens and different segments of gynoecium, ovary, style and stigma. 21. Abaxial view of a young stamen with enlarging cells indicated by arrows, note the position of the nectary. 22. mature nectary with arrows indicating the position of the stomata. 23. Stamens and petals position in developing flower bud. 24. Petals elongation and expansion in a developed flower bud overlaping each other. SE = Sepal; AN = Anther; OV = Ovary, Sl = Style; St = Stigma; N = Nectary, P = Petal

clearly that petal initiation occurred in the last steps of the floral bud development (Fig. 17). This is in contradiction with Polowick and Sawahney (1986) data on *B. napus* floral organ development. In the specimens from 9-leaved plantlets, elongation of the gynoecium was pursued at the same rate as that of the long stamens (Fig. 18) but at the same time the petal primordia are still in the primary stages of development. In this specimen and in the precedent samples the beginning of an invagination and visible furrow formation at the tip of gynoecium was demonstrated, which became a deeper when the plantlets reached the 9 leaved stage.

Chronology of organ development: In confirmation of the observation made by Polowick and Sawhney (1986) we have also observed that the initiation of sepals begins with the abaxial sepal primordium emergence and this is shortly followed by the appearance of the adaxial and the two lateral sepals (Fig. 11 and 12). Enlargement of the sepals continue, causing them to stay close to the apex. Abaxial and adaxial sepals cover the two lateral sepals. The abaxial one covers completely the three others specially at the top end of the sepals and meristem dome (Fig. 13 and 19). Due to this situation the observation of the other flower organ development demands the removal of the sepals and additional preparations in flower buds. The previously stained specimens should be re-manipulated by dissecting and cutting away the sepals in order to study the inner hidden organs. This possibility may be considered as the main advantage of the epi-illumination technique in comparison to the SEM technique. Stomata were clearly observable in the abaxial surface of abaxial sepal where it curved over the meristem dome. It seems that there is a zonal distribution of stomata on the sepal abaxial surface (Fig. 13).

Synchrony in stamens and gynoecium primordial emergence were observed in our studies (Fig. 14) which is in contradiction with previous studies on *Brassica* flower organ development (Polowich and Sawhney, 1986). Due to the comparable size of stamens and gynoecium it seems that the elongation rate in these two organs proceeds in the same rate (Fig. 15). Stamen development begins with the inception of the four long primordia and shortly after, the emergence of the two small stamen primordia can be seen. These two stamens were clearly situated in a lower position as compared with the long ones. At maturity, the anther size of both type of stamens are comparable and they seems to have the same pace of growth (Fig. 20). A distinct pattern of cells were observed in the abaxial and inferior parts of each anther. These cells are globular and bulbous (Fig. 21). The first stage of the nectary primordium initiation between the primary filaments of long stamens was observed in Fig. 21. In the later stages of nectary primordium development the stomata formation and zonal position of stomata were clearly observed on the superior parts of nectarines (Fig. 22). At the 7-leaved plantlets, gynoecium is fully initiated and the beginning of invagination is clearly observable (Fig. 14). This invagination becomes deeper and more profound in the later steps (Fig. 15 and 16).

Based on our data the petal initiation was not discernible before the beginnings of invagination in the gynoecium (Fig. 14), but in the subsequent steps, petal primordia were clearly perceptible (Fig. 15 and 16). The petals were situated in the same level as the short stamens and their growth rate were slow in comparison with the growth rate of other organs (Fig. 16 and 17). This slow rate of growth was recaptured in the next steps of development and they finally reached the same size as the other inner organs of flowers and consequently they cover the stamens and gynoecium (Fig. 23 and 24). These results are in fair concordance with those of Polowick and Sawhney (1986) about the development of some organs such as sepals and stamens. While, there are some contradictions in the chronology of gynoecium and petals development.

CONCLUSIONS

Floral initiation in *Brassica napus* L. consists of a multi-step process that is characterized by morphological changes of the apex. Organogenesis of reproductive in rapeseed could be successfully studied by epi-illumination light microscopy instead of SEM. Epi-illumination light microscopy offers an adequate instrumental capacity for the detection of protodermal cell division during ontogeny of the flower. Taken the results together, developmental patterns of meristematic regions and flower primordial initiation could be studied with more detail with this procedure. Furthermore, the studied material could be re-manipulated for more detailed investigation of inner organs by dissection and separation of outer organs. Present results revealed that the order of whorls inception in *Brassica* do not follow the standard ABC model. Since the petal primordia initiates at the last step of floral development, then we conclude that this species is categorized as an uncommon model for general developmental pattern. Also, some aspects of our findings about the order of petal whorl formation do not concord with other data from previous studies. Therefore, floral ontogeny in *Brassica napus* L. has an explicit complexity and exceptional pattern.

REFERENCES

Chakravarti, S.C., 1953. Organization of shoot apex during the ontogeny of *Brassica campestris* L. Nature, 171: 223-222.

Charlton, W.A., A.D. Macdonald, U. Posluszny and C.P. Wilkins, 1989. Additions to the technique of epi-illumination light microscopy for the study of floral and vegetative apices. Can. J. Bot., 67: 1739-1743.

Cronquist, A., 1968. The Evolution and Classification of Flowering Plants. Thomas Nelson and Sons Ltd., London.

Hayward, H.E., 1938. The Structure of Economic Plants. The Macmillan Co., N.Y.

Orr, R.A., 1978. Inflorescence development in *Brassica campestris* L. Am. J. Bot., 65: 466-470.

Polowick, P.L. and V.K. Sawhney, 1986. A scanning electron microscopic study on the initiation and development of floral organs of *Brassica napus* (CV. WESTAR). Can. J. Bot., 73: 254-260.

Posluszny, U., M.G. Scott and R. Sattler, 1980. Revisions in the technique of epi-illumination light microscopy for the study of floral and vegetative apices. Can. J. Bot., 58: 2491-2495.

Prenner, G., 2003. A developmental analysis of the inflorescence and the flower of *Lotus corniculatus* (Fabaceae-Loteae). Mitt. Naturwiss. Ver. Steiermark, 133: 99-107.

Robbelen, G., R.K. Downey and A. Ashri, 1989. Oil Crops of the World. MC Graw-Hill, New York, pp: 157-183.

Rosenberg, S.M. and H.T. Bonnett, 1983. Floral organogenesis in *Nicotiana tabacum*: A comparison of two cytoplasmic male sterile cultivars with a male fertile cultivar. Am. J. Bot., 71: 1347-1363.

Sadik, S. and J.L. Ozbun, 1967. Histochemical changes in the shoot tip of cauliflower during floral. Can. J. Bot., 45: 955-959.

Sattler, R., 1968. A technique for the study of floral development. Can. J. Bot., 46: 720-722.

Sattler, R., 1973. Organogenesis of Flower: A Photographic Text-atlas. Univ. of Toronto Press, Toronto.

Smyth, D.R., J.L. Dowman and E.M. Meyerowitz, 1990. Early flower development in *Arabidopsis*. The Plant Cell, 2: 755-767.

Tucker, S.C., 1982. Inflorescence and flower development in the Piperaceae. II. Inflorescence and flower development of *Piper*. Am. J. Bot., 69: 743-752.

Tucker, S.C., 1984. Unidirectional organ initiation in leguminous flowers. Am. J. Bot., 71: 1139-1148.

Tucker, S.C., 1985. Initiation and development of inflorescence and flower in *Anemopsis californica* (Saururaceae). Am. J. Bot., 72: 20-31.