MORPHO-HISTOLOGICAL ASPECTS OF ADVENTITIOUS SHOOT FORMATION WITHOUT PLANT GROWTH REGULATORS IN SEED EXPLANTS OF *Capsicum annuum* L., AND IMPACT OF PRECULTURE ON REGENERATION

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Abstract. The physiological state of plant material is the crucial endogenous factor at the explant choice for plant regeneration. The phases of germination characterised by various, following each other biochemical and developmental processes can affect the organogenesis capability. This research examined the morphological and anatomical events during the early stages of organogenesis and plant regeneration in explants derived from seeds of *Capsicum annuum* L., cv. Bryza preincubated under high humidity conditions from 0 to 6 days and next cultured on MS medium without PGRs. The early stage of *de novo* shoot formation reminded leaf differentiation *in planta*. First the leaves began to differentiate as spherical and tongue shaped structures from epidermis and subjacent layers of the explants about the 7th day of culture. In some cases nearly at the base of previously formed leaf and even on its petiole one or two leaves as well as shoot apex in their axils were induced thereby forming young shoot which underwent elongation and whole plant regeneration after 2 subculturing. More advanced developmentally structures of adventitious shoot were obtained while prolonging preculture duration. This was the favourable effect on the shoot differentiation, their elongation and plant regeneration as seed submitted preculture for 3, 4, 5 days however, seeds not treated with preculture revealed the best response as regard to shoot primordium formation at the earliest stage.

Key words: pepper, shoot morphogenesis, germination, histogenesis, leaf formation *in vitro*, plant regeneration without PGRs, shoot apical meristem

INTRODUCTION

Biotechnological breeding methods of the agronomic and horticultural important species have been implemented already for many years however, for new genotypes

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usually it is necessary to elaborate or modify the regeneration protocol especially for *in vitro* recalcitrant plant species such as genus *Capsicum* [Kothari et al. 2010, Segui-Simarro et al. 2011].

The first regenerated plants of pepper were achieved over 30 years ago [Gunay and Rao 1978]. Ever since various factors influencing regeneration process such as the source of explant [Agrawal et al. 1989, Ebida and Hu 1993], explant position [Fari and Czako 1981, Gatz and Rogozińska 1994], age and developmental stage of donor explants [Ramirez-Melagon and Ochoa-Alejo 1996], genotyp [Diaz et al. 1988, Ochoa-Alejo and Ireta-Moreno 1990], as well as media components and growth regulator combinations [Phillips and Hubstenberger 1985] and also gamma ray exposure [Sripichitt et al. 1988] have been carried out. Although complete pepper plant regeneration has been achieved from protoplasts [Saxena et al. 1981, Diaz et al. 1988] and through direct or indirect organogenesis [Gunay and Rao 1978, Fari 1986], as well as via somatic embryogenesis [Harini and Lakshmi Sita 1993, Khan et al. 2006] and even on the way of androgenesis [George and Narayanaswamy 1973], its efficiency is not still satisfactory. This is often because of shoot elongation disorders resulting from formation of the leaf-like structures or rosette shoots instead of normal ones [Szasz et al. 1995, Kothari et al. 2010].

Among the pepper regeneration protocols one of the most beneficial manner in view of natural formation of shoot bud and plant regeneration has been reported by Ezura at al. [1993]. In that procedure halves of mature seeds as explants were used and any exogenous plant growth regulators were not required. Under these conditions, induction and shoot bud formation arise in more similar way to shoot morphogenesis in nature, which allows comparison of the shoot bud developmental abnormalities following exogenous plant growth regulators (PGRs) application. Mature seeds and embryos are frequently used as primary explants due to their high morphogenetic potential helpful for regeneration. With regard to the seed explants, imbibition and further changes involving germination might also influence upon their regeneration abilities [Pierik 1987]. In the current research the early stages of the adventitious shoot formation in the half seed explants of *Capsicum annuum* L., cv. Bryza, taking morphological characterisation and changes on the histological level into consideration, were analysed. Moreover, the effect of preculture i.e. duration of the seed maintenance under high humidity conditions upon the shoot primordium induction and further development to shoots and the whole plant regeneration was also carried out.

MATERIAL AND METHODS

Pepper seeds (*Capsicum annuum* L.) cv. Bryza were obtained from PlantiCo and used as plant material in this study. First the seeds were immersed in 70% ethanol for about 10 seconds and subsequently they were surface sterilised with a 4% (w/v) sodium hypochlorite with a few drops of Tween 20 for 7 minutes. After the washing in sterile distilled water (3 times), the seeds were put on Petri dishes with filter paper soaked water (equal volume of water for each dish and parafilm to eliminate evaporation were used) for preculture which lasted from 0 to 6 days. The imbibed seeds in one day intervals and the ones not treated with preculture were cut into two parts; the part containing

the proximal part of hypocotyl and the radicle was used as a constitute explant. Halves of the seeds were horizontally placed into the MS medium [Murashige and Skoog 1962] without plant growth regulators. The pH of the medium was adjusted to 5.8 and solidified by 0.7% (w/v) Difco Bacto Agar.

To present the quantity and quality aspects of shoot formation within the half of seed explants, the induced and formed structures were classified on the base of their morphology into 4 categories (fig. 1). Moreover, every week during one month of culture, number of structures per explant were determined by using a light stereomicroscope (Zeiss Stemi 2000-C). After four weeks of culture segments of hypocotyls in length about 2 mm, including structures mentioned above, were cut from primary explants and subcultured on the MS medium also without PGRs for three weeks in purpose of the shoot elongation. Subsequently elongated shoots were counted, isolated from explants and next individually inoculated on the fresh MS medium aimed rooting. Explant cultures were maintained under a 16 h photoperiod (day light fluorescent illuminations; 35 μmol $m^{-2} s^{-1}$) and temperature 25ºC.
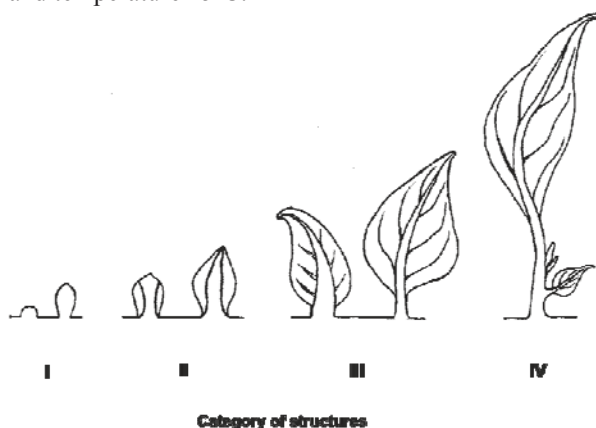

Category of structures

Fig. 1. Category of structures distinguished within seed pepper explants during adventitious shoot formation: I – leaf primordium of the spherical-shaped and tongue-shaped, II – leaf primordium with lamina and main midrib but yet without petiole, III – leaf with the distinct division into lamina and petiole, IV – young adventitious shoot with visible shoot tip

To study the early histological events of adventitious shoot bud initiation samples from organogenic zone were collected at different time of culture (at 0; 3; 7; 14 and 21 days) and fixed in formaldehyde-based fixative containing formaldehyde 5%, acetic acid 5%, 70% ethanol 90%. Tissue samples were dehydrated in graded acetone series, embedded in paraffin, sectioned serially by rotary microtome at the thickness of 10 μm. The sections were stained with hematoxylin [Darlington and La Cour 1960], examined under Nikon Eclipse E-600 light microscope and photographed with Nikon digital camera (DXM 1200). The experiment was repeated twice and each preculture variant was evaluated in 48 explants. Statistical analyses were performed using analysis variance ANOVA MANOVA for two-factorial experiments with Tukey's multiple range post host testing to determine significance between groups at α = 0.05.

RESULTS AND DISCUSSION

The early stages of the adventitious shoot bud formation in the pepper seed explants without plant growth regulators (PGRs), morphologically and histologically reminded typical stages of the leaf development *in vivo* but with regard to examined explants in the absence of shoot apical meristem (SAM). Taking also under consideration the influence of seed preculture upon regeneration ability, all macroscopically visible structures around the cut surface of the seed explant that is hypocotyl during the first month of culture, were grouped into four categories which simultaneously presented developmental stages of leaf and adventitious shoot (fig. 1).
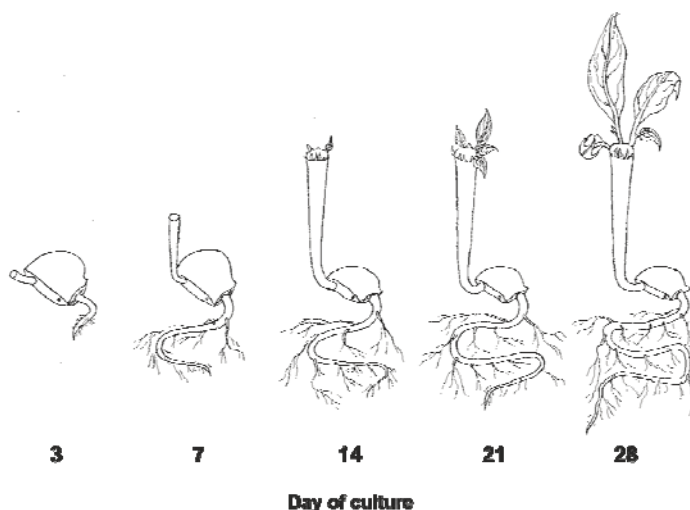

Fig. 2. The phase of organogenesis into the half seed explants of pepper on MS medium in the absence of the plant growth regulators (PGRs) during 4 weeks of culture

In the first week of explant incubation, hypocotyl elongation and root development were observed (fig. 2). Moreover, in the explants except those derived from zero-day preculture, leaf primordia formation around the cut explant surface took place. Cell divisions starting the leaf primordium differentiation were observed already after 3 days of culture however, they were not initiated from procambium (phot. 1a A) being primary meristem but from peripheral tissues of the explant (phot. 1a B). Those divisions were mainly periclinal in adjacent layer as well as anticlinal within epidermis and led during a few days to formation of protrusions representing the early stage of the leaf development, macroscopically visible as spherical- and tongue-shaped structures (category I structures), (fig. 1, phots 1ab D–E, 2 A–B). They were at times connected already with vascular system of the explant (phot. 1ab). The first cell divisions involved in adventitious shoot formation without PGRs in the half seed explants occurred just a few days after inoculation like in the different other types of pepper explants [Fari 1986, Kothari et al. 2010] however, treated exogenously with PGRs. It was confirmed that the ability of explant cells to acquire competence taking place during the initial phase of dedif-

ferentiation depends first of all on the regulation by the pathways of auxin and cytokinin signalling [Duclerq et al. 2011], which are the key factor to start *de novo* organogenesis. Moreover, within individual explants from 5th and 6th day preculture, except the leaf primordium induction, their further development already followed as a result of mitotic activity into the above mentioned layers of the explant (phot. 1ab), giving structures II category (fig. 1, phots 1b F, 2 C). These structures corresponded with the stage of the forming leaf lamina in which main midrib was visible.

After two weeks of the seed explant culture, some of leaf primordia developed into young leaves consisting of two distinct parts – lamina and petiole (fig. 1, phots 1b G, 2 D–E). The initiation of periclinal and antyclinal divisions in epidermis and subsequently subepidermis, next arising of spherical- and tongue-like shape outgrowths, lamina expansion and petiole within studied explants resemble the first proliferating leaf founder cells, the initiation of leaf primordium, and next polar differentiation of primordium into leaf lamina (from distal region) and petiole (from region between proximal leaf base and distal lamina) in dicots [Harper and Freeling 1996, Scanlon 2000].

During the third week of culture, on the explants precultured from 2 to 6 days, sometimes it was observed how at the base of petiole previously formed leaf, other leaf and in some cases even two leaves (phot. 2 F) began to differentiate and furthermore in the axils of those leaves, shoot apex also was initiated. Shoot apex probably was originated from the zone between new formed leaves which consisted of the cells with distinct meristematic features such as relatively small size, isodiametric in shape with prominent, central localised nucleus and dense cytoplasm (phot. 1b H). The place where such leaves were differentiated, additionally was protruded leading to formation of separately young shoot (phot. 1b H). So at the end of the fourth culture week within explants from all variant duration of preculture, structures of 4 classified categories were present simultaneously, except from zero-day preculture explants where mainly the first and second categories of structures were differentiated; leaves on these explants were observed sporadically and young shoots were not at all (fig. 3 D).

In the case of the studied seed explants, typical shoot bud formation with shoot apex and pair of leaf primordia, like it was within hypocotyls or cotyledonary explants of the same *Capsicum* cultivar [Gatz 2002] treated with PGRs, has not been observed. It seems that initially alone leaf primordia were differentiated from meristematic zones (phot. 1ab) without distinctly separated shoot apical meristem (SAM). The same independence of the leaf formation in other plant species has been reported for example in *Taraxacum* root, both leaf primordia and leaves were initiated *de novo* in the absence of adventitious SAM [Bowes 1976], in *Begonia* where leaf-like appendages arise from young leaf [Sattler and Maier 1977], in *Arabidopsis* SAM was regenerated from the axils of the youngest prior produced leaves [Keller et al. 2006]. Also in others pepper varieties not typical adventitious shoot organization, particularly after applying the high cytokinin concentration has been reported [Delis et al. 2005, Mezghani et al. 2007]. It is worth noting that in some cases within studied half seed explants at the base the first regenerated leaf and even within its petiole the second and third new leaves were initiated without visible shoot tip. Likely in *Zeylanidium subulatum* the second leaf was formed from meristematic cells not being SAM nearby adjacent to the first leaf [Imaichi et al. 2005]. Similar results with relation to the formation a few first leaves of bud before
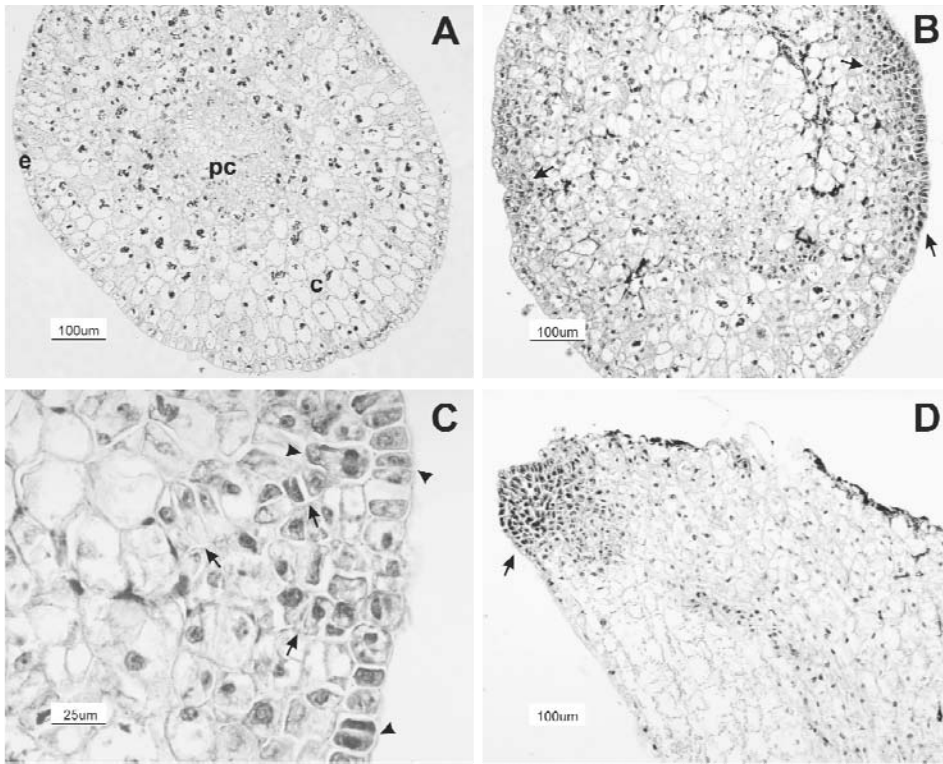
A – Transverse section of the cut place (hypocotyl) of explant before inoculation on the medium
B – Transverse section showing mitotic activity of the cells (arrows) in epidermis and subjacent layers after 3 days of culture
C – Anticlinal (arrow heads) and periclinal (arrows) divisions within epidermis and cortex cells after 3 days in transverse section
D – Spherical shaped structure (category I structure) being the earliest stage of leaf primordium differentiation in the longitiudinal section after 7 days of culture
e – epidermis, co – cortex, pc – procambium

Phot. 1a. Histological aspects of *de novo* shoot organogenesis in the half seed explants of *Capsicum annuum* L., cv. Bryza cultured on the MS medium without plant growth regulators (PGRs)
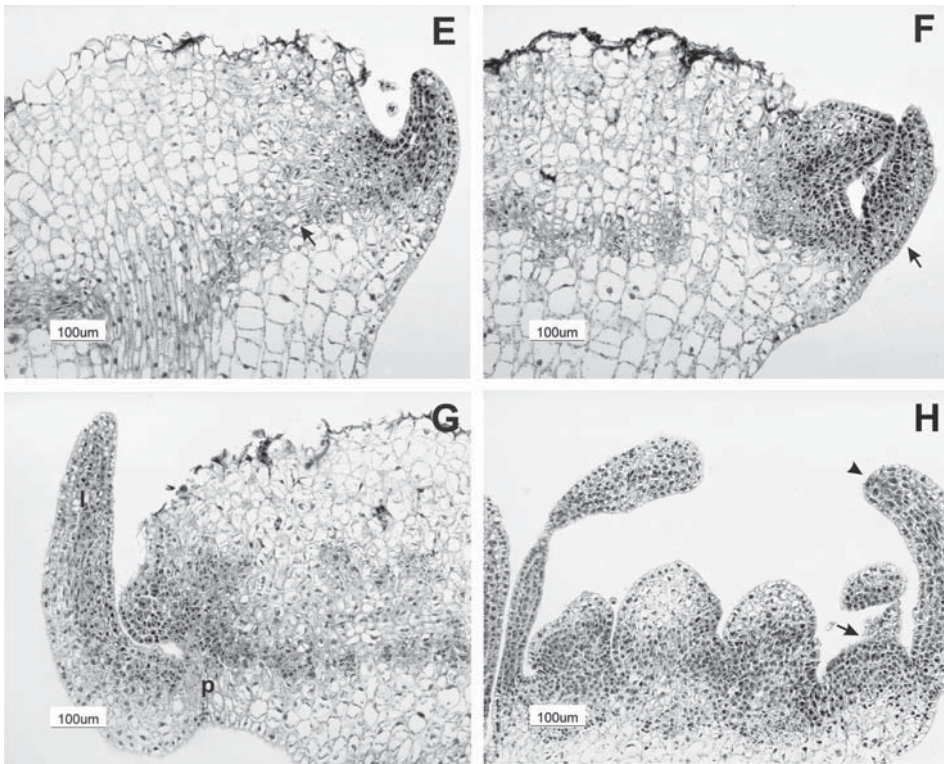
E – Leaf primordium of the tongue shaped (category I structure) with connection zone between itself and main vascular system of explant in longitiudinal section after 14 days of culture

F – Longitiudinal section showing leaf primordium with starting of petiole formation (arrow) (category II structure) after 14 days of culture

G – The view of the young leaf (category III structure) with lamina (l) and petiole (p) in the longitiudinal sections after 21 days of culture

H – Longitiudinal section showing the beginning of the shoot formation – leaf differentiating (arrow) nearly at the base of the other early formed leaf after 21 day of culture

e – epidermis, co – cortex, pc – procambium

Phot. 1b. Histological aspects of *de novo* shoot organogenesis in the half seed explants of *Capsicum annuum* L., cv. Bryza cultured on the MS medium without plant growth regulators (PGRs)
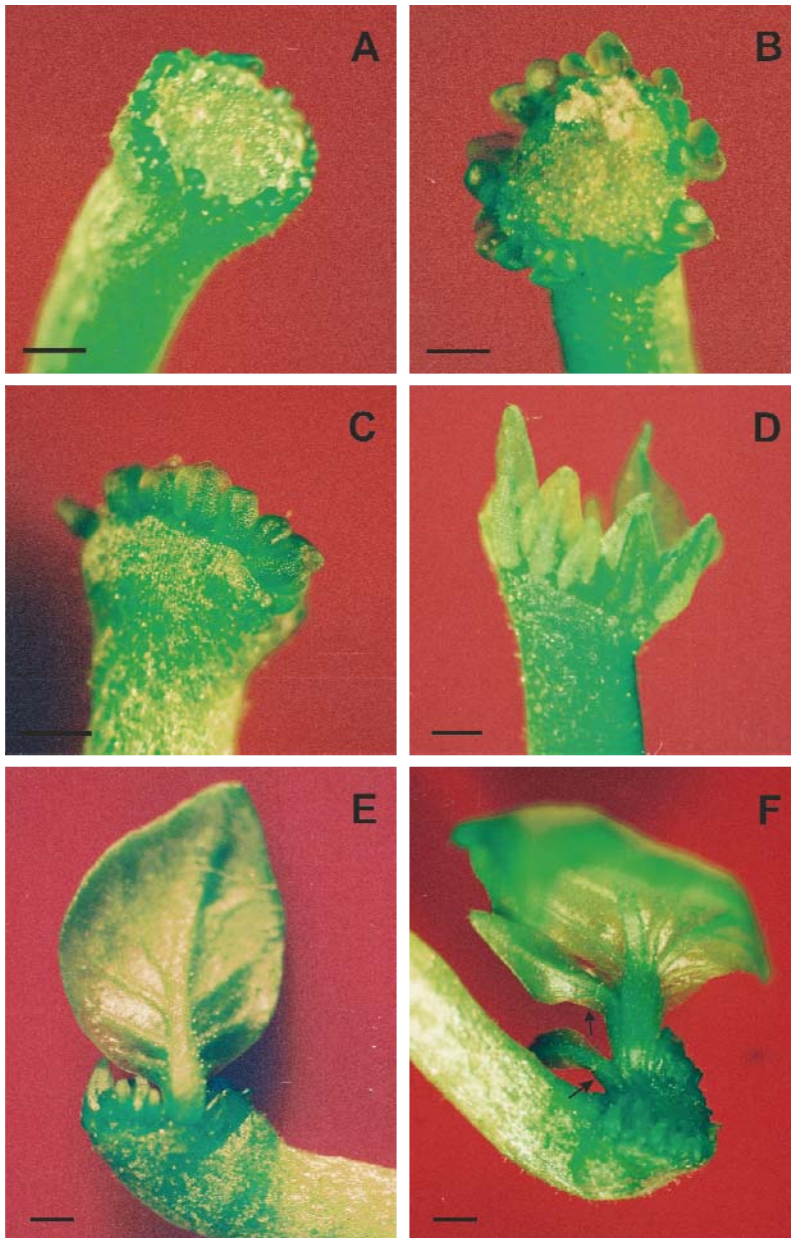
Phot. 2. Morphology of structures (I–IV categories) from different stages of *de novo* shoot forma-
tion within seed explants of *Capsicum annuum* L. cv. Bryza: A – spherical-shaped leaf
primordia, B – tongue-shaped leaf primordia, C – leaf primordia as laminas with marked
main midrib, D – young leaves with developing lamina and petiole, E – leaf, F – forming
young shoot (arrows indicate additional leaves nearly at the base and petiole primarily
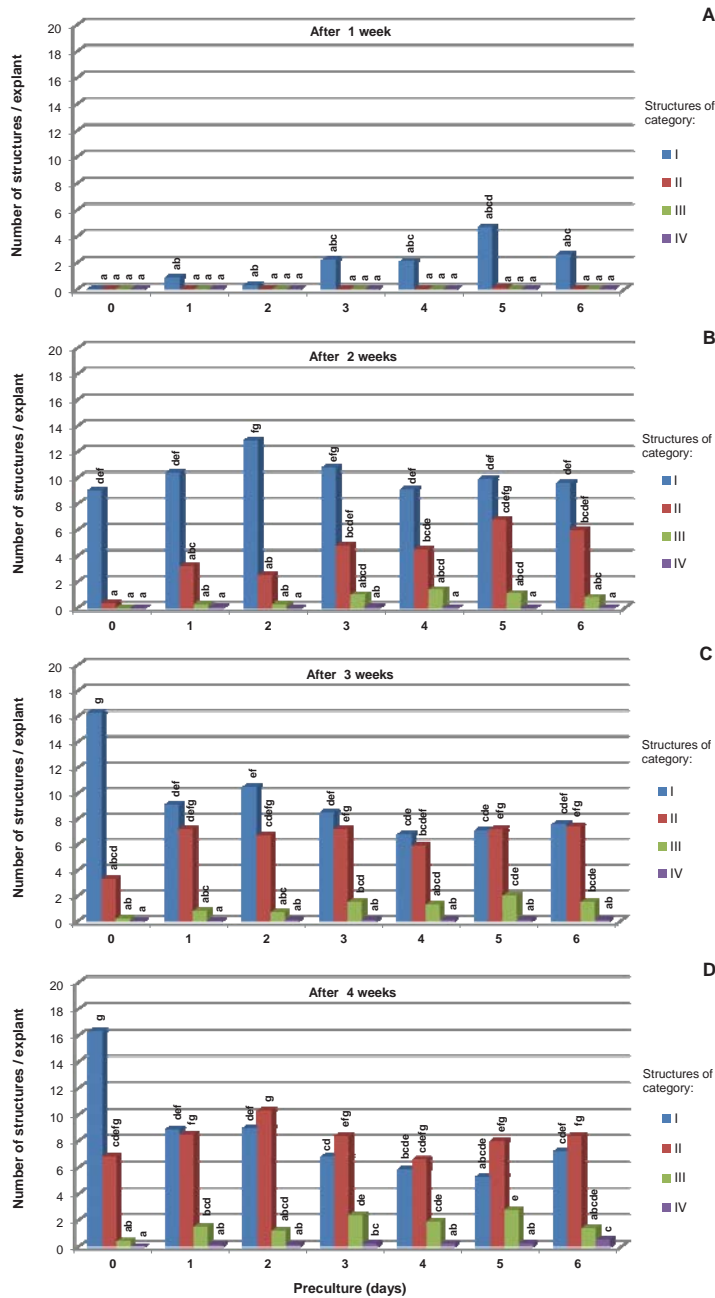regenerated leaf). Bar = 10 mm

Fig. 3. The effect of preculture on the differentiation efficiency of the structures (I-IV category) within pepper seed explants after the first (A), second (B), third (C) and fourth (D) weeks of culture on the MS medium without PGRs. The same letters mark not significant differences by the Tukey's test at α = 0.05

SAM arising and the pattern of positioning for new leaf by newly formed leaf primordia in axils of *Nasturtium officinale* have been reported [Selker and Lyndon 1996]. Additionally, the possibility of leaf primordium development after switching off the expression of *PINHED* gene, connected with normal activity of shoot meristem in *Arabidopsis* has been shown [Mc Connell and Barton 1995].

Generally in this study the applied preculture i.e. the maintaining of pepper seeds in the high humidity conditions from 0–6 days, has essential effect upon both the efficiency of shoot induction (with the certain exception), as well as the achievement of their further development and eventually upon the whole plant regeneration. Explants, in which the preculture treatment was applied, underwent water uptake by imbibitions (phase I of seed germination), in the different progress of the reserve mobilisation (phase II of seed germination), and in some cases also the last stage of germination characterised by the radicle elongations. The longer time of preculture, the greater is the phytohormonal activity essential for germination as well as the availability to ATP and carbon skeletons for *de novo* synthesis of proteins resulting prior hydrolysis of storage compounds in the case of pepper mainly protein [Blasiak et al. 2006] in a smaller degree – starch [Lijun et al. 2012].

As regards to the preculture effect on the shoot induction, estimation of its efficiency can be based indirectly on the quantity of the category I structures thus the highest number of these structures were recorded on the explants derived from zero-day preculture, and it amounted 16.3 leaf primordia per explant in the third week of culture (fig. 3 C). In spite of the fact that explants from 0 day preculture variant revealed the greatest ability to initiate shoot organogenesis although further development of leaf primordia initially into leaves and subsequent shoots was insignificant (fig. 3). It may result in these explants, which were halves of the seeds, the initiation of germination did not follow yet hence at the moment of explant isolation seeds were in dormancy, and even the imbibition did not occur that is essential germination phase to set in motion the biochemical and physiological events [Nonogaki et al. 2010].

The lower efficiency of the shoot bud induction was noticed in the case of explants from 4th, 5th and 6th day preculture, on the other hand from these preculture variants, the most structures of I category at the earliest that is in the first week of culture were achieved (fig. 3). It may point that exactly within those explants, faster although slightly lower number of cells acquired the competence to respond to the hormonal stimulus of probably residing endogenic auxins [De Klerk et al. 1997]. Those auxins could have activated *Aux/IAA*, *GH3* and *SAUR* gene families essential for that phase of shoot organogenesis [Elhiti and Stasolla 2012]. In seed *Pinus silvestris* L., free IAA released from conjugates stored can be present during imbibition but first of all it occurs prior to the radicle emergence [Ljung et al. 2001]. In swelling *C. annuum* seeds from 5th and 6th day preculture variants radicle protrusion were visible, and it is possible that those seeds from 4th and even 3rd day preculture have been already characterised by initial radicle emergence.

On the contrary to the category I structures mentioned above, number of these ones of II-IV category, gradually have been increasing during the first month of culture and at the end of it, II category of structures were the highest (9.9 leaf primordia/explants) in the case of explants derived from the 2nd day of preculture, young leaves (category III

structure) from the 5th day of preculture (2.6 leaf/explant) and IV-category structure (0.4 shoot buds/explant) at prolonged one day of the preculture treatment (fig. 3 D). The duration of the preculture effected on physiological activity and degree of radicle development. In actively dividing cells of the root meristem, cytokinins are synthesized and transported into xylem to different regions, particularly to meristematic tissues of shoot apex. As early mentioned, within explants from 3 to 6 day preculture it was started and continued the phase III of germination characterised by radicle protrusion resulting in meristematic activity. Thus in the explant cut place i.e. hypocotyl it might be suitably high level of cytokinins in relation to auxins that was favourable for the shoot regeneration. It is supported by recently proposed model of the interaction between auxin and cytokinin in cell proliferation and new organ formation [Su et al. 2011]. In that model high ratio of cytokinin/auxin as shown may promote shoot organogenesis by the transcription factors *WUSCHEL* (*WUS*) expression which is essential to SAM formation *de novo*.
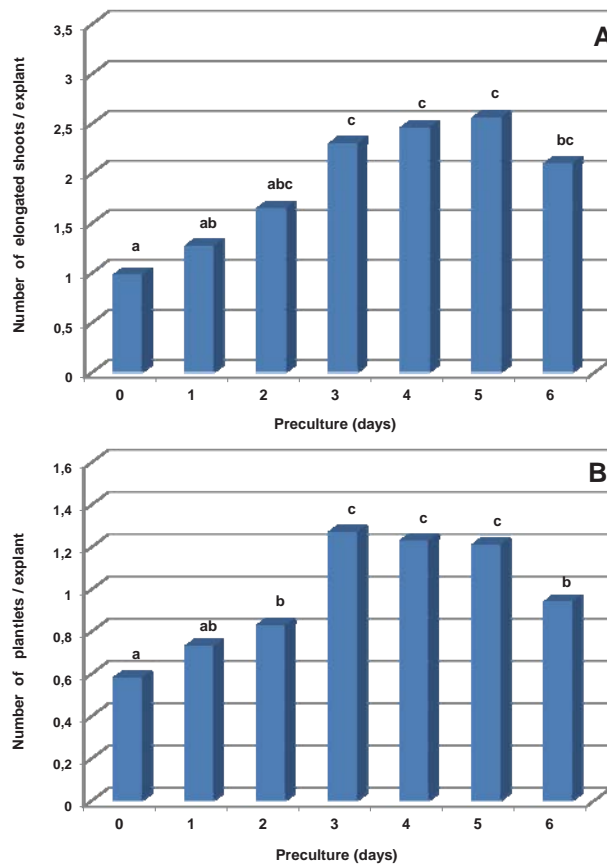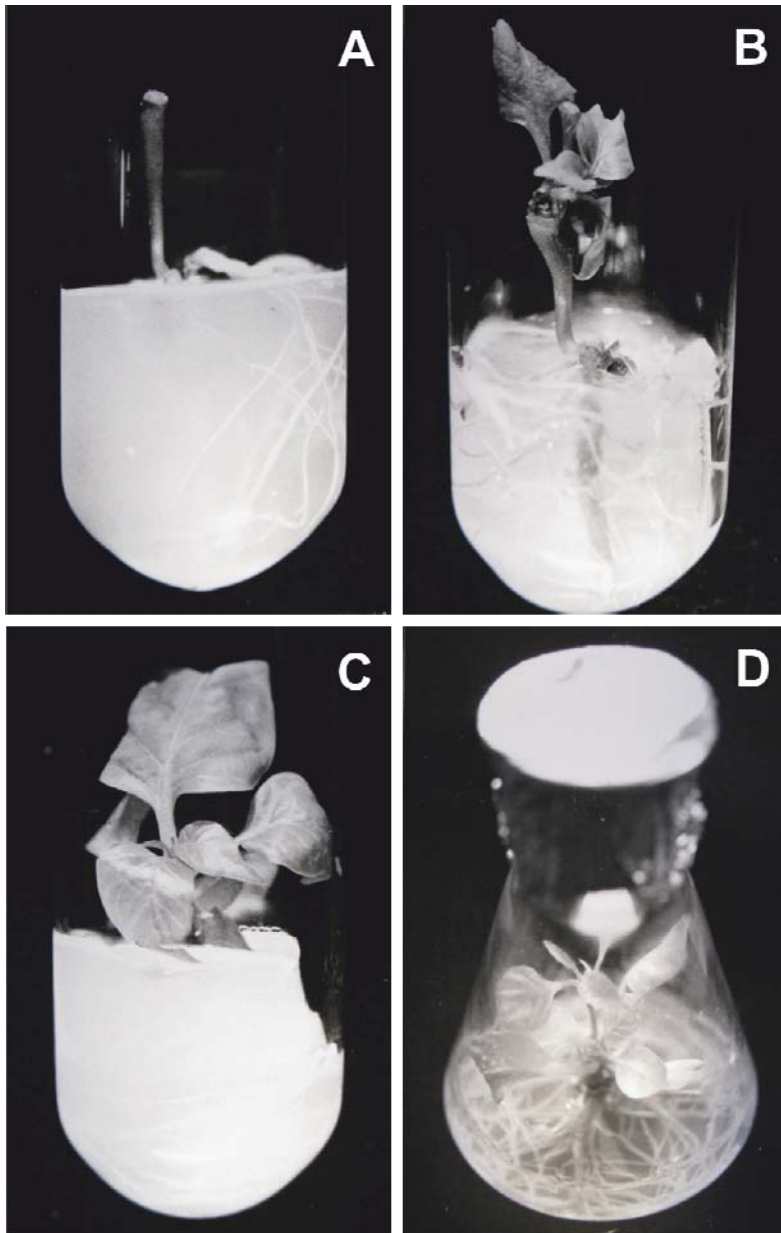

Fig. 4. The effect of preculture on the elongation of young shoots (A), and their rooting thereby plantlet regeneration (B). The same letters mark not significant differences by the Tukey`s test at $\alpha = 0.05$

Phot. 3. The stages of plantlet regeneration from pepper seed explants without PGRs: A – the elongated hypocotyl on which surface the formation of first structures – leaf primordia began after 7 days of culture, B – young shoots present within primarily explants after 4 weeks, C – shoot elongation from subcultured segment of hypocotyl after subsequent 3 weeks of culture, D – rooting of the excised shoot and plantlet achievement during 3 weeks

As a result of 4-week subculturing of cut hypocotyl fragment, including young shoot, leaves and their primordia, only not numerous ones (fig. 4 A) underwent further differentiation, shoot elongation and forming morphologically normal shoots (phot. 3 C). It may be associated with a meristem of young shoot and leaves that are the main places of biosynthesis of auxin which transported downward to the places of shoot differentiation may cause the apical dominance in relation to existing leaf primordia and leaves, like in plant stem auxin inhibits outgrowth of axillary buds. However, as recently has been reported, auxin has not directly effected on this process but through interaction with strigolactones or cytokinins as well [Dun et al. 2009]. Moreover, it seems that strigolactones which can be present in different places of plant, those ones produced in the shoot have suppressive effect on the bud outgrowth [Ruyter-Spira et al. 2013].

Like at the first stage of pepper regeneration (induction and young shoot formation), during the first subculturing aimed shoot elongation, the essential influence of preculture duration upon the elongation efficiency was also noticed (fig. 4 A). The capacity of elongation was markedly increased with increasing of preculture periods, except six-day preculture variant at which little decrease of yield of elongated shoot buds followed. This may result from the fact that seed explants from variants of longer time preculture contained higher number of structures more advanced in development. The subculture enabled these ones most similar developmentally to shoots, further differentiation and elongation as well as rizogenesis thereby full plant regeneration during the second passage after previous isolation such shoots from explants. Also in that last step of plantlet regeneration relevant to root formation (phot. 3 D), the efficiency of achieved microseedlings was the highest, similarly as during shoot elongation in the case of variants of 3th, 4th and 5th preculture day (fig. 4 B) which is, to some extent, the consequence of obtaining higher quantity of alone shoots already before the rooting.

In *Capsicum* sp. exogenous PGR application causes that leaf like structures and rosette shoots often occur and they are the essential factor limiting the plant regeneration. Therefore the quite rapid method of plantlet achievement (phots 3 A–D) without exogenic PGRs, free from morphological disorders of regenerated shoots appears to be promising for Polish pepper cultivars however, it requires further study to increase its relatively low efficiency.

CONCLUSIONS

1. The early stages of shoot formation began with the leaf differentiation in the 7th day of culture without visible on the histological level typical SAM. Instead the induced leaf primordia developed into leaves and subsequently from several leaves nearly lying, the shoot was formed and its tip arose probably in the leaf axil just after three culture weeks.

2. In initiating adventitious shoots participated epidermal as well as subepidermal cortical layers and not procambium (primary meristem) also existing in the explant. The first cell divisions within those layers have become already in the third day since explant inoculation.

3. Preculture involved in different phase of seed germination has significant effect on the efficiency especially the induction of shoots, their elongation and finally plantlet regeneration which was higher for seed explants from 3, 4, 5 and 6 days of preculture. Those preculture variants corresponded to the end of phase II and phase III of pepper seed germination.

REFERENCES

Agrawal S., Chandra N., Kothari S.L., 1989. Plant regeneration in tissue cultures of pepper (*Capsicum annuum* L. cv. Mathania). Plant Cell Tiss. Org. Cult. 16, 47–55.

Blasiak J., Kuang A., Farhangi C.S., Musgrave M.E., 2006. Roles of intra-fruit oxygen and carbon dioxide in controlling pepper (*Capsicum annuum* L.) seed development and storage reserve deposition. J. Amer. Soc. Hort. Sci 131, 164–173.

Bowes B.G., 1976. Polar regeneration in excised roots of *Taraxacum officinale* weber: a light and electron microscopic study. Ann. Bot. 40, 423–432.

Darlington G.D., La Cour L.F., 1960. The handling of chromosomes. London. Unwin Ltd. 163.

De Klerk G.J., Arnholdt-Schmitt B., Lieberei R., Neumann K.H., 1997. Regeneration of roots, shoots and embryos: physiological, biochemical and molecular aspects. Biol. Plant. 39, 53–66.

Delis M., Garbaczewska G., Niemirowicz-Szczytt K., 2005. Differentiation of adventitious buds from *Capsicum annuum* L. hypocotyls after co-culture with *Agrobacterium tumefaciens*. Acta Biol. Cracov. Ser. Bot. 47, 193–198.

Diaz I., Moreno R., Power J.B., 1988. Plant regeneration from protoplasts of *Capsicum annuum*. Plant Cell Rep. 7, 210–212.

Duclercq J., Sangwan-Norrel B., Catterou M., Sangwan R.S, 2011. *De novo* shoot organogenesis: from art to science. Trends Plant Sci. 16, 597–606.

Dun E.A., Brewer P.B., Beveridge C.A., 2009. Strigolactones: discovery of the elusive shoot branching hormone. Trends Plant Sci. 14, 364–372.

Ebida A.I.A., Hu C.Y., 1993. *In vitro* morphogenetic responses and plant regeneration from pepper (*Capsicum annuum* L. cv. Early California Wonder) seedling explants. Plant Cell Rep. 13, 107–110.

Elhiti M., Stasolla C., 2012. *In vitro* shoot organogenesis and hormone response are affected by the altered levels of *Brassica napus* meristem genes. Plant Sci. 190, 40–51.

Ezura H., Nishimiya S., Kasumi M., 1993. Efficient regeneration of plants independent of exogenous growth regulators in bell pepper (*Capsicum annuum* L.). Plant Cell. Rep. 12, 676–680.

Fari M., Czako M., 1981. Relationship between position and morphogenetic response of pepper hypocotyl explant cultured *in vitro*. Sci. Hortic. Amsterdam 15, 207–213

Fari M., 1986. Pepper (*Capsicum annuum* L.). In: Biotechnology in Agriculture and Forestry, vol. 2, Crops, Bajaj Y.P.S. (ed.). Springer Verlag Berlin, Heidelberg, New York, Tokyo 345–362.

Gatz A., Rogozińska J., 1994. *In vitro* organogenetic potential of cotyledon and leaf explants of *Capsicum annuum* L. cv. Bryza. Acta Soc. Bot. Pol. 63, 255–258.

Gatz A., 2002. Histological changes during the adventitious shoot formation in seedling explants of pepper (*Capsicum annuum* L.) cultured *in vitro*. Acta Soc. Bot. Pol. 71, 269–274.

George L., Narayanaswamy S., 1973. Haploid *Capsicum* through experimental androgenesis. Protoplasma 78, 467–470.

Gunay A.L., Rao P.S., 1978. *In vitro* plant regeneration from hypocotyl and cotyledon explants of red pepper (*Capsicum*). Plant Sci. Lett. 11, 365–372.

Harini I., Lakshmi Sita G., 1993. Direct somatic embryogenesis and plant regeneration from immature embryos of chilli (*Capsicum annuum* L.). Plant Sci. 89, 107–112.

Harper L., Freeling M., 1996. Studies on early leaf development. Curr. Opin. Plant Biot. 7, 139–144.

Imaichi R., Hiyama Y., Kato M., 2005. Leaf development in the absence of a shoot apical meristem in *Zeylanidium subulatum* (*Podostemacea*). Ann. Bot. 96, 51–58.

Keller T., Abbott J., Moritz T., Doerner P., 2006. *Arabidopsis REGULATOR OF AXILLARY MERISTEMS1* controls a leaf axil stem cell niche and modulates vegetative development. Plant Cell. 18, 598–611.

Khan H., Siddique I., Anis M., 2006. Thidiazuron induced somatic embryogenesis and plant regeneration in *Capsicum annuum.* Biol. Plant 50, 789–792.

Kothari S.L., Joshi A., Kachhwaha S., Ochoa-Alejo N., 2010. Chili peppers. A review on tissue culture and transgenesis. Biotech. Adv. 28, 35–48.

Lijun O., Zhuqing Z., Xiongze D., Xuexiao Z., 2012. Seed micromorphology and germination characteristics of wild and cultivated pepper strains. Plant Syst. Evol. 298, 1211–1216.

Ljung K., Ostin A., Lioussanne L., Sandberg G., 2001. Developmental regulation of indole-3-acetic acid turnover in Scot pine seedlings. Plant Physiol. 125, 464–475.

Mac Connell J.R., Barton M.K., 1995. Effect of mutations in the *PINHEAD* gene of *Arabidopsis* on the formation of shoot apical meristems. Dev. Genet. 16, 358–366.

Mezghani N., Jemmali A., Elloumi N., Gargouri-Bouzid R., Kintzios S., 2007. Morpho-histological study on shoot bud regeneration in cotyledon cultures of pepper (*Capsicum annuum*). Biologia 62, 704–710.

Murashige T., Skoog F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol. 15, 473–497.

Ochoa-Alejo N., Ireta-Moreno L., 1990. Cultivar differences in shoot-forming capacity of hypocotyls tissues of chili pepper (*Capsicum annuum*) cultured *in vitro*. Sci. Hortic. 42, 261–269.

Nonogaki H., Bassel G.W., Bewley J.D., 2010. Germination – still a mystery. Plant Sci., 179, 574–581.

Phillips G.C., Hubstenberger J.F., 1985. Organogenesis in pepper tissue cultures. Plant Cell. Tiss. Org. Cult. 4, 261–269.

Pierik R.L.M., 1987. *In vitro* culture of higher plants. Martinus Nijhoff Publishers Dordrecht.

Ramirez-Malagon R., Ochoa-Alejo N., 1996. An improved and reliable chili pepper (*Capsicum annuum* L.) plant regeneration method. Plant Cell. Rep. 16, 226–231.

Ruyter-Spira C., Al-Babili S., Van der Krol S., Bouwmeester H., 2013. The biology of strigolactones. Trends Plant Sci. 18, 72–83.

Sattler R., Maier U., 1977. Development of epiphyllous appendages of *Begonia hispida* var. *cucullifera* – implications for comparative morphology. Can. J. Bot. 55, 411–425.

Saxena P.K., Gill R., Rashid A., Maheshwari S.C., 1981. Isolation and culture of protoplasts of *Capsicum annuum* L. and their regeneration into plants flowering *in vitro*. Protoplasma 108, 357–360.

Scanlon J.M., 2000. Developmental complexities of simple leaves. Curr. Opin. Plant Biol. 3, 31–36.

Selker J.M.L., Lyndon R.F., 1996. Leaf initiation and de novo pattern formation in the absence of an apical meristem and pre-existing patterned leaves in watercress (*Nasturtium officinale*) axillary explants. Can. J. Bot.74, 625–641.

Segui-Simarro J.M., Corral-Martinez P., Parra-Vega V., Gonzalez-Garcia B., 2011. Androgenesis in recalcitrant solanaceous crops. Plant Cell. Rep. 30, 765–778.

Sripichitt P., Nawata E., Shigenaga S., 1988. The effect of exposure dose and dose rate of gamma radiation on i*n vitro* shoot forming capacity of cotyledon explants in red pepper (*Capsicum annuum* L. cv. Yatsufusa). Jap. J. Breed. 38, 27–34.

Su Y.H., Liu Y.B., Zhang X.S., 2011. Auxin-cytokinin interaction regulates meristem development. Mol. Plant 4(4), 616–625.

Szasz A., Nervo G., Fari M., 1995. Screening for *in vitro* shoot-forming capacity of seedling explants in bell pepper (*Capsicum annuum* L.) genotypes and efficient plant regeneration using thidiazuron. Plant Cell. Rep. 14, 666–669.

MORFOLOGICZNO-HISTOLOGICZNE ASPEKTY FORMOWANIA PĘDÓW PRZYBYSZOWYCH BEZ REGULATORÓW WZROSTU W EKSPLANTATACH NASION *Capsicum annuum* L. ORAZ WPŁYW PREKULTURY NA REGENERACJĘ

Streszczenie. Stan fizjologiczny materiału roślinnego jest kluczowym endogennym czynnikiem przy wyborze eksplantatu do regeneracji roślin. Fazy kiełkowania, charakteryzujące się różnymi następującymi po sobie biochemicznymi i rozwojowymi procesami, mogą mieć wpływ na zdolności do organogenezy. W pracy tej przebadano morfologiczne i anatomiczne przemiany podczas wczesnych etapów organogenezy i regeneracji roślin w eksplantatach pochodzących z nasion *Capsicum annuum* L. odmiany Bryza, które wstępnie inkubowano w wilgotnych warunkach od 0 do 6 dni, a następnie utrzymywano w kulturach na pożywce MS bez regulatorów wzrostu (R.W.). Wczesne stadia formowania pędów *de novo* przypominały różnicowanie liścia *in planta*. Pierwsze liście zaczęły różnicować się jako struktury o kształcie sferycznym i języczkowatym w epidermie i warstwach leżących tuż pod nią około 7 dnia kultury eksplantatów, w niektórych przypadkach bardzo blisko podstawy wcześniej powstałego liścia, a nawet na jego ogonku. Jeden a nawet dwa liście i wierzchołek pędu w ich kątach były indukowane, formując w ten sposób młody pęd, który ulegał elongacji i pełnej regeneracji do rośliny po 2 pasażach. Wydłużając czas prekultury, otrzymywano struktury bardziej zaawansowane rozwojowo. Korzystny wpływ na różnicowanie pędów, ich elongację i regenerację roślin zaznaczył się, kiedy nasiona poddano prekulturze przez 3, 4 i 5 dni, chociaż nie traktowane ujawniły najlepszą odpowiedź odnośnie formowania zawiązków pędowych w ich najwcześniejszym etapie.

Słowa kluczowe: papryka, histogeneza, regeneracja roślin bez regulatorów wzrostu, merystem wierzchołkowy pędu