DEVELOPMENTAL AND MORPHOLOGICAL STUDIES OF THE SUSPENSOR AND COLEORHIZAE IN SOME TAXA OF THE SUBFAMILY MIMOSOIDEAE (FABACEAE)

CHUAN QI SHI, MEI LIU*, XIN XIN ZHANG, XIN YU CHENG AND CHEN WANG

Harbin Normal University, College of Life Science and Technology, Key Laboratory of Molecular Cytogenetic and Genetic Breeding of Heilongjiang Province, 150025 Harbin, P.R. China
*Correspondence author's e-mail: m.r.liu@126.com; Tel: +86 451 8806 0576; Fax: +86 451 8806 0575

Abstract

The embryo development of *Mimosa pudica* and the seedling development of *Acacia farnesiana*, *Albizzia julibrissin*, *Leucaena glauca* and *Mimosa pudica* were studied by wax method in this paper. The results showed that *M. pudica* has a suspensor composed of about ten cells which degenerate and integrate into the embryo proper during the cotyledons development. The coleorhiza in *M. pudica* is from the residual suspensor cells and the cells produced by periclinal and anticlinal divisions of the meristematic cells in the embryo development. The coleorhiza in the four studied taxa is broken by the elongation of the primary root, withdraws and finally falls off during the seedling development.

Key words: Morphological studies, Suspensor, Coleorhizae, Mimosoideae (Fabaceae).

Introduction

Fabaceae (or Leguminosae) is an important economic family which includes three subfamilies: Mimosoideae (actinomorphic flower with valvate arrangement of petals), Caesalpinioideae (zygomorphic flower with imbricate arrangement of petals) and Faboideae (or Papilionoideae, zygomorphic flower with papilionaceous corolla) (Takhtajan, 2009; Angiosperm Phylogeny Group, 2009). The studies on the family Fabaceae were very extensive, including physiology and ecology (e.g. Azim *et al*., 2013; Li *et al.*, 2013), embryology and morphology (e.g. Rodriguez-Pontes, 2007, 2008; Al-Ghamdi & Al-Zahrani, 2010; De-Paula & Oliveira, 2012) and molecular systematics (Shinwari *et al*., 2014). Kawashima & Goldberg (2010) described the suspensors occur in some taxa of Fabaceae (e.g. *Glycine max* & *Lathyrus angustifolia*). Suspensor is an important structure which derives from the basal cell of the 2-celled proembryo in the embryo development. It suspends the embryo, transfers nutrients and secrets hormones to the embryo proper (Johansen, 1950; Kawashima & Goldberg, 2010). There are various suspensors in Fabaceae, for example, in Faboideae: *Phoseolus coccineus* has a massive suspensor (Nagl, 1974) and *Glycine* have long-lived suspensors (Li *et al*., 1990; Zhang *et al*., 1990; Wang *et al*., 1991); in Caesalpinioideae: *Cassia occidentalis* has a uniseriate filament suspensor with about five short cells (Pantulu, 1951) and the suspensors of *Senna corymbosa* (Rodriguez-Pontes, 2007) and the subtribe Cassiinae (De-Paula & Oliveira, 2012) are poor development; in Mimosoideae: *Leucaena glauca* has a filamentous suspensor (Dnyansagar, 1949, 1954). However, there is no suspensor differentiation in some taxa of this family, e.g. *Sophora secundiflora* (Guigurd, 1881) of Faboideae; *Ceratonia siliqua*, *Gymnocladus dioica* (Guigurd, 1881) and *Cassia tora* (Rau, 1951) of Caesalpinioideae and *Acacia baileyana* (Newman, 1934), *A. farnesiana* (Narasimhachar, 1948) and *Mimosa pudica* (Guigurd, 1881; Narasimhachar, 1951) of Mimosoideae. Lersten (1983) comprehensively summarized the suspensors in Fabaceae and referred there is no suspensor

in the 21 species of Mimosoideae except *Leucaena glauca*. Johansen (1950) divided Mimosoideae into two categories based on if the species with a suspensor differentiation and suggested that the type of embryo development without suspensor is of the *Trifolium* variation *Onagrad* type. But Prakash (1987) stated that the *Onagrad* type with a suspensor differentiation predominates in Mimosoideae.

Coleorhiza is a sheath-like structure covering the radicle of grasses and *Hemerocallise* (Liliaceae) in monocotyledon. It protects the primary root during the seedling development (e.g. Avery, 1930; Brown, 1960; Sutton & Tinus, 1983; Feng *et al*., 2003; Zhang *et al*., 2013). It is partially homologous with those of the cycads in gymnosperm which is related to the remaining distal part of the suspensor (e.g. Dorety, 1908; Chamberlain, 1919, 1935; Pant & Sing, 1991; Robbertse *et al*., 2011). Juncosa (1984) indicated that the coleorhiza is formed from the outer cortical meristem in the seedling development of *Bruguiera exaristata* (Rhizophoraceae). However, Raven *et al*. (1992) suggested that there is no coleorhiza in dicotyledons.

In order to investigate the origin of the sheath-like structure occurred on the primary root during the seed germination of *Mimosa pudica*, we deeply studied on the embryo development of it and the seedling development of *Acacia farnesiana*, *Albizzia julibrissin*, *Leucaena glauca* and *Mimosa pudica*. Our aims are to reveal if *M. pudica* has a suspensor in its embryo development, the relationship between the suspensor and the sheath-like structure, and to discuss the taxonomic value of the suspensor in Mimosoideae.

Materials and Methods

Young fruits and seeds of *Mimosa pudica* were collected from Harbin Normal University Botanic Garden (Shi 2801, HANU). Seeds of *Acacia farnesiana* were from Kunming (Cai 12cs4736, KUN), *Albizzia julibrissin* from Hangzhou (Liu s. n. HANU) and *Leucaena glauca* from Guangzhou (Liu s. n. HANU).

The young fruits of *Mimosa pudica* were fixed in Carnoy's fluid (alcohol: acetic acid= 3: 1) for 4-6 h, then stored in 70% alcohol and its seeds were dipped into 95°C water for 1 min (Zhang *et al*., 2010). A part of testa of *Acacia farnesiana*, *Albizzia julibrissin* and *Leucaena glauca* was cut and all of them were cultivated at 22°C. The different developmental periods of the hypocotyl and primary root were taken, fixed and stored by FAA (70% alcohol: formalin: acetic acid= 90: 5: 5). According to Li (1987), these materials were stained by Hematoxylin, dehydrated by a series concentration of alcohol, penetrated by xylene and then embedded in wax. The sections were made at 6-8 μm.

The sections were observed and photographed by Olympus BX51 microscope and digital camera DP70. The germinating seeds were photographed by Olympus digital camera SP350.

Result

The suspensor differentiation of *Mimosa pudica*: The first division of the zygote of *Mimosa pudica* is transverse and forms a 2-celled proembryo with an apical cell and a basal cell. Both of them are similar in size (Figs. 1, 2). The longitudinal division of the apical cell nucleus appears before the transversal division of the basal cell nucleus (Fig. 3) and the proembryo is T-shaped with 4-nucleolate (Fig. 4). The proembryo develops further from T-shape to small globe. There are some deeply stained and slightly larger cells at the micropyle end which are divided from the basal cell and quite different from the embryo proper cells (Fig. 5). These cells are not increased obviously in number when the large globular proembryo is formed from the quick division of the embryo proper cells (Fig. 6).

The heart-shaped embryo with a handle-shaped structure is formed when the two cotyledonal primordia develop. This structure connected the embryo proper with the endosperm is the suspensor which is composed of about ten cells from the divisions of slightly larger cells at the micropyle end (Fig. 7). The undeveloped suspensor has no obvious boundary with the embryo proper. In the torpedo-shaped embryo stage, the suspensor with a polyploid nucleus (about 3 μm in size) is slightly bent and deviates from the micropyle and penetrates into the endosperm (Fig. 8).

The coleorhizae of *Mimosa pudica* and related taxa: The embryo rotates 90° in the further development of cotyledons. The apex cells of the suspensor gradually degenerate, but the distal residual cells integrate into the embryo proper. There are 8-10 layers of light color and oblong shaped cells which are formed by the anticlinal and periclinal divisions of a group of meristematic cells which are deep color and square shape in the front of the radicle during the embryo maturation (Figs. 9, 13). The inner layers form the root cap and the outer layers of cells connected with the cells of the residual suspensor form a sheath-like structure, i.e. the coleorhiza, around the radicle. The cells of the coleorhiza and root cap are similar in shape (Figs. 9, 14).

The radicle of *Mimosa pudica* breaks through the seed coat firstly with the hypocotyl elongating rapidly. The transition zone which consists of the enlarged parenchyma cells around the vascular tissue is found between the hypocotyl and the primary root. Between the transition zone and the root apex, the coleorhiza surrounds the primary root (Figs. 10, 11). With further elongation and enlargement of the primary root, the coleorhiza is broken, withdraws and eventually falls off (Fig. 11b), and some inner cells of the remained coleorhiza stay on the root cap. The similar development pattern was also observed during the seedling of *Albizzia julibrissin* (Fig. 12). The coleorhiza in *Acacia farnesiana* and *Leucaena glauca* are so extremely similar to *Albizzia julibrissin* that did not demonstrate here.

Discussion

The suspensor development of *Mimosa pudica* and its taxonomic signification: Lersten (1983) suggested that the size and shape of the suspensor vary in different angiosperm species, even in the different stages of embryo. In the early stages of the embryo development, the division of suspensor cells is faster than embryo proper cells. The suspensor develops well in the heart stage, and then gradually degenerates as cotyledons develop, finally obliterates before the mature embryo formation. Guigurd (1881) and Narasimhachar (1951) described the division of cells is irregular after the 4-celled stage and forms a pear-shaped massive type embryo with no suspensor differentiation in the embryo development of *Mimosa pudica*. However, our study showed that a handle-like suspensor is present in the globular stage, and then a handle-shaped suspensor is formed in the heart stage. The most developed suspensor is differentiated in torpedo stage and degenerates during the cotyledons development. Finally, it integrates into the embryo proper and participates in the coleorhiza formation. The later development and poor differentiation of the suspensor in *M. pudica* is similar to that in *Leucaena glauca*.

Johansen (1950) suggested that there are two categories of embryogeny in Mimosoideae according to the suspensor differentiation, one with and the other lacking a suspensor. Based on this classification, Narasimhachar (1951) indicated that the embryo development of *M. pudica* is of the *Trifolium* variation *Onagrad* type without a suspensor. However, *Cercis siliquastrum* was regarded as an example for the category with a suspensor differentiation (Johansen, 1950), and this species was placed in Caesalpinioideae (Takhtajan, 2009), but not Mimosoideae. Johansen's classification may be not perfect. Our study showed *Mimosa pudica* has an easily neglected suspensor which has about ten cells and no obvious boundary with the embryo proper. The embryo development of *M. pudica* is *Onagrad* type rather than *Trifolium* variation *Onagrad* type.
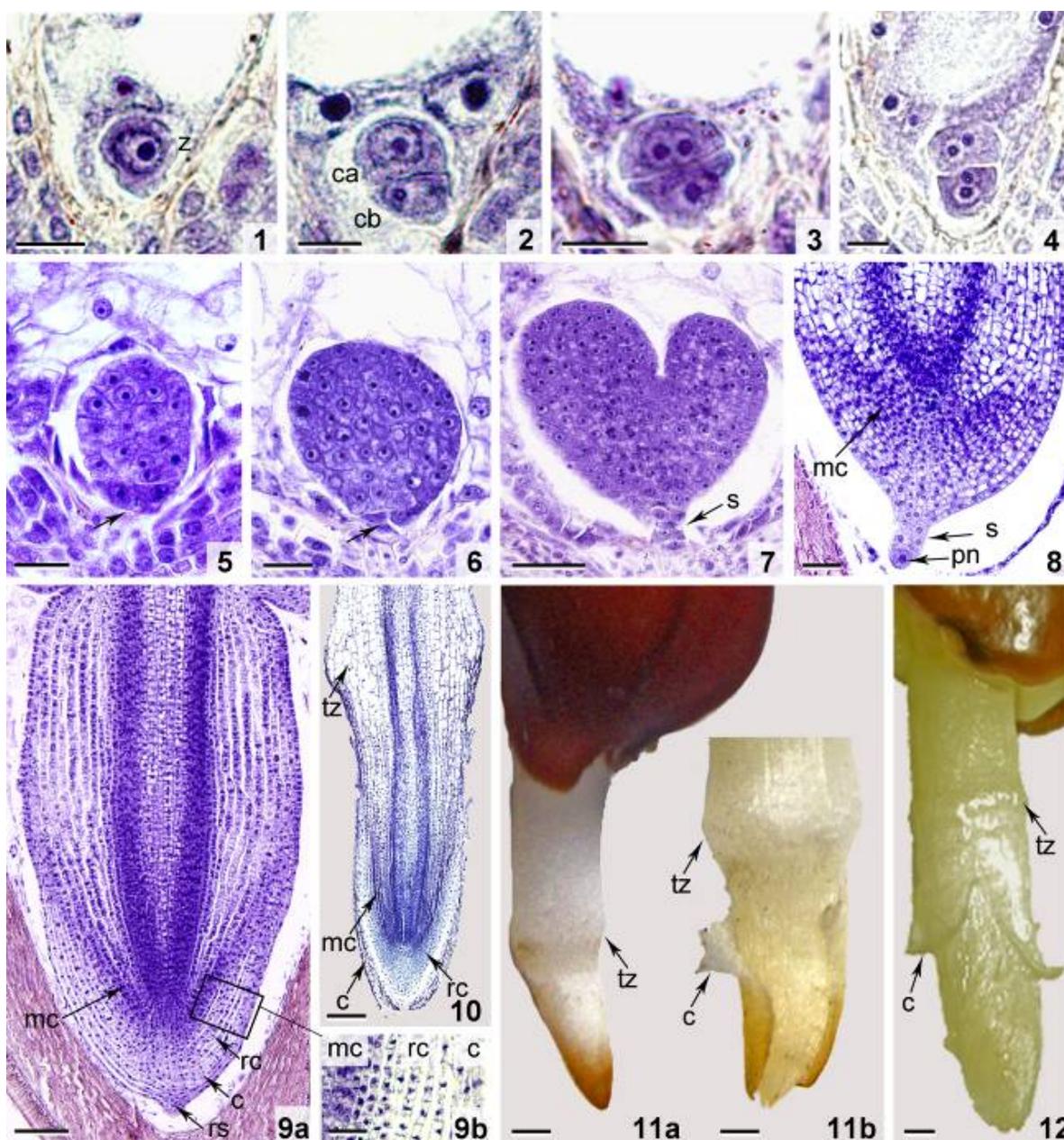
Figs. 1-12. The embryo and seedling development of *Mimosa pudica* (1-11) and *Albizzia julibrissin* (12). 1. Zygote. 2. 2-celled proembryo. 3. Vertical division of the apical cell. 4. Transverse division of the basal cell to form a 4-nucleolate proembryo. 5. Small globular proembryo. 6. Large globular proembryo. 7. Heart-shaped embryo. 8. Torpedo-shaped embryo. 9a. Mature embryo. 9b. Meristematic cells, root cap and coleorhiza. 10. Longitudinal section of primary root. 11a. Primary root. 11b. Broken coleorhiza. 12. Coleorhiza of *Albizzia julibrissin*. ca = apical cell; cb = basal cell; c = coleorhiza; mc = meristemic cells; pn = polyploid nucleus; s = suspensor; rc = root cap; rs = residual suspensor; tz = transition zone; z = zygote. Scale bars: 1-4 = 10 μm; 5, 6, 9b = 20 μm; 7, 8 = 50 μm; 9a = 100 μm; 10 = 500 μm; 11, 12 = 1 mm.

The origin of the coleorhiza in Mimosoideae: The coleorhiza in cycads originates from the suspensor (e.g. Dorety, 1908; Chamberlain, 1919, 1935; Pant & Sing, 1991) and Robbertse *et al*. (2011) stated a coleorhiza does exist in cycad embryo and derives from the distal part of the suspensor. The coleorhiza in grasses is also relative to the suspensor (e.g. Avery, 1930; Brown, 1960; Sutton & Tinus, 1983), though Feng *et al*. (2003) described that the upper portion of the hypoblast contributes to the formation of the coleorhiza in *Zea mays*. The coleorhiza in *Hemerocallise* derives from the residual suspensor, the proper next to the suspensor, and the cells from the meristematic cellular band occurred in the radicle (Zhang *et al*., 2013). Juncosa (1984) showed the coleorhiza in *Bruguiers exaristata* is formed by lobes of the outer cortical meristem during the seedling development, which are same with the meristematic cells of *Mimosa pudica*. Narasimhachar (1948) described the embryo development

of *Acacia farnesiana* has no suspensor differentiation, but all the four studied species (including *A. farnesiana*) should originate from the cells of the residual suspensor and the cells from the outward divisions of the meristematic cells as their coleorhizae appear in the seedling development. The coleorhiza in Mimosoideae is partially homologous to those in cycads, *Bruguiers exaristata*, grasses and *Hemerocallise*.

The coleorhiza occurs in Myrtaceae (Baranov, 1957) and Juncosa (1984) described that *Bruguiers exaristata* has a coleorhiza and he suggested a coleorhiza is present in various dicotyledons, but it usually originates late in embryo development. Raven *et al*. (1992) stated there is no coleorhiza in dicotyledons, and Robinson *et al*. (2008) argued the structure in Myrtaceae reported by Baranov (1957) is not coleorhiza but hypocotyl hairs. Our study showed that the coleorhiza exists in some species of Mimosoideae which protects the primary root during the early seedling development and also in *Astragalus membranaceus* and *Arachis hypogaea* (Fabaceae) (pers. obs.). But it needs to study further the origination of the coleorhiza and if it is wide present in dicotyledons.
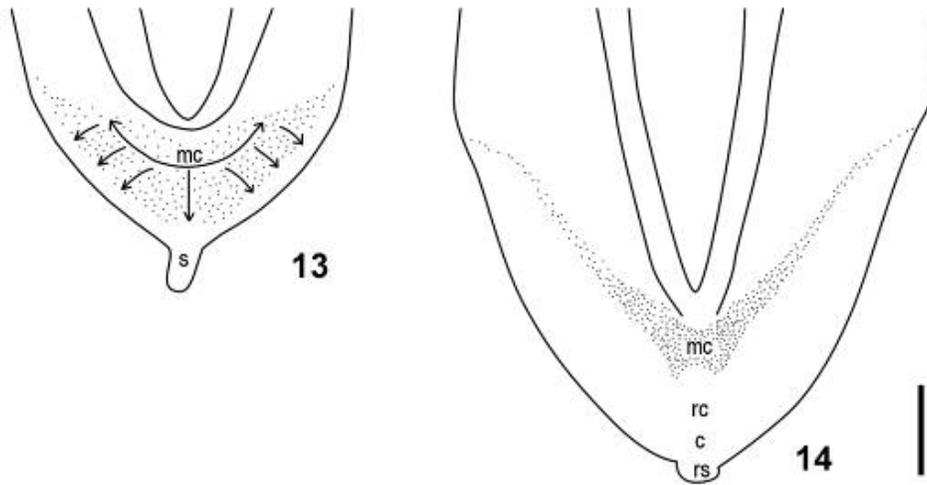
Figs. 13, 14. Simplified illustrations of the divisions of the meristematic cells to show the origin of the coleorhiza in *Mimosa pudica*. 13. The periclinal and anticlinal divisions of the meristematic cells showed by arrows. 14. The coleorhiza derives from the residual suspensor cells and the cells from the divisions of meristematic cells. c = coleorhiza; mc = meristematic cells; s = suspensor; rc = root cap; rs = residual suspensor. Scale bar: 13 = 70 μm; 14 = 100 μm.

Conclusion

The study on the embryo development of *Mimosa pudica* and the seedling development of *Acacia farnesiana*, *Albizzia julibrissin*, *Leucaena glauca* and *Mimosa pudica* showed that a suspensor dose exist in *M. pudica*, though it is poor and late development. There is no obvious boundary between the suspensor and the embryo proper. The suspensor degenerates and integrates into the embryo proper during the mature embryo formation. The coleorhiza derives from the cells of the residual suspensor and cells closed to the residual suspensor which are from the divisions of meristematic cells. It is broken and finally falls off with the elongation of the primary root in the seedling development. The coleorhiza in Mimosoideae is partially homologous with those in cycads, *Bruguiers exaristata*, grasses and *Hemerocallise*.

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References

Al-Ghamdi, F.A. and R.M. Al-Zahrani. 2010. Seed morphology of some species of *Tephrosia* Pers. (Fabaceae) from Saudi Arabia Identification of species and systematic significance. *Feddes Repert*., 121(1-2): 59-65.

Angiosperm Phylogeny Group. 2009. An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG. *Bot. J. Linn. Soc.*, 161(2): 105-121.

Avery, G.S. 1930. Comparative anatomy and morphology of embryos and seedlings of maize, oats, and wheat. *Bot. Gaz.*, 89(1): 1-39.

Azim, M.R., Q. Mahmood and S.S. Ahmad. 2013. Risk evaluation of brick kiln smoke to kidney bean (*Phaseolus vulgaris*). *Pak. J. Bot.*, 45(4): 1403-1406.

Baranov, P.A. 1957. Coleorrhiza in Myrtaceae. *Phytomorphology*, 7: 237-243.

Brown, W.V. 1960. The morphology of the grass embryo. *Phytomorphology*, 10(3): 215-223.

Chamberlain, C.J. 1919. *The living cycads*. University of Chicago Press, Chicago.

Chamberlain, C.J. 1935. *Gymnosperms. Structure and evolution*. University of Chicago Press, Chicago.

De-Paula, O.C. and D.M.T. Oliveira. 2012. Seed ontogeny of *Chamaecrista* and its systematic implications in Cassiinae (Leguminosae, Caesalpinioideae). *Plant Syst. Evol*., 298(9): 1659-1669.

Dnyansagar, V.R. 1949. Embryological studies in the Leguminosae. I. A contribution to the embryology of *Leucaena glauca* Benth. *J. Ind. Bot. Soc.*, 28: 95-107.

Dnyansagar, V.R. 1954. Embryological studies in the Leguminosae. X. Supplementary observations on the development of the endosperm and embryo in *Leucaena glauca* Benth. and *Mimosa hamata* Willd. *J. Ind. Bot. Soc.*, 33: 433-442.

Dorety, H.A. 1908. The seedling of *Ceratozamia. Bot. Gaz.*, 46: 203-220.

Feng, J.H., X.B. Xu, X.D. Liu, C.L. Zhang, X.L. Liang and W.C. Wu. 2003. Embryogenesis, germination, structure and cotyledon dimorphism of *Zea mays* embryo. *Acta. Bot. Sinic.*, 45(6): 712-723.

Guignard, L. 1881. Recherches d'embryogénie Végétale Comparée I. Legumineuses. *Ann. Sci. Nat. Bot.*, 12: 5-166.

Johansen, D.A. 1950, *Plant Embryology*. Waltham, Mass.

Juncosa, A.M. 1984. Embryogenesis and developmental morphology of the seedling in *Bruguiera exaristata* Ding Hou (Rhizpphoraceae). *Amer. J. Bot.*, 71(2): 180-191.

Kawashima, T. and R.B. Goldberg. 2010. The suspensor: not just suspending the embryo. *Trends Plant Sci.*, 15(1): 23-30.

Lersten, N.R. 1983. Suspensors in Leguminosae. *Bot. Rev.*, 49: 233-257.

Li, H.R., G.W. Tian and J.H. Shen. 1990. Investigation on embryology of soybean. *Natural Sci. J. Harbin Nor. Uni.*, 6(3): 61-69.

Li, J.F., S.Q. Zhang, P.H. Huo, S.L. Shi and Y.Y. Miao. 2013. Effect of phosphate solubilizing rhizobium and nitrogen fixing bacteria on growth of alfalfa seedlings under P and N deficient conditions. *Pak. J. Bot.*, 45(5): 1557-1562.

Li, Z.L. 1987. *The techniques of making plant sections*. Science Press, Beijing.

Nagl, W. 1974. The *Phaseolus* suspensor and its polytene chromosomes. *Z. Pflanzenphysiol.*, 73(1): 1-44.

Narasimhachar, S.G. 1948. A contribution to the embryology of *Acacia farnesiana* L. (Willd.). *Proc.: Plant Sci.*, 28(4): 144-149.

Narasimhachar, S.G. 1951. An embryological study of *Mimosa pudica* Linn. *Proc. Ind. Acad. Sci.*, 33(4): 192-198.

Newman, I.V. 1934. Studies in the Australian acacias: IV. The life history of *Acacia baileyana* F.V.M. Part II, Gametophytes, fertilization, seed production and germination, and general conclusion. *Proc. Linn. Soc.*, 59: 277-313.

Pant, D.D. and R. Sing. 1991. Unusual orthotropous germination in seeds of *Cycas rumhii* Miq. and the morphological nature of cycad coleorhiza. *Plant Sci. Bull.*, 2: 9-14.

Pantulu, J.V. 1951. Studies in the Caesalpiniaceae. II. Development of the endosperm and embryo in *Cassia occidentalis* L. *J. Ind. Bot. Soc.*, 30: 95-99.

Prakash, N. 1987. Embryology of the Leguminosae. *Advances in legume systematics*, 3: 241-278.

Rau, M.A. 1951. The endosperm in some species of *Cassia* L. *Svensk Bot. Tidskr*, 45: 516-522.

Raven, P.H., R.F. Evert and S.E. Eichhorn. 1992. *Biology of plants*. (5th Ed) Worth Publishers, New York.

Robbertse, H.P.J., N. Grobbelaar. and E. du Toit. 2011. Origin of the coleorhiza in cycad seedlings and its structural homology with that of the Poaceae. *Bot. Rev.*, 77(1): 1-10.

Robinson, R.W., P.I. Boon, N. Sawtell, E.A. James and R. Cross. 2008. Effects of environmental conditions on the production of hypocotyl hairs in seedlings of *Melaleuca ericifolia* (swamp paperbark). *Aust. J. Bot.*, 56(7): 564-573.

Rodriguez-Pontes, M. 2007. Development of megagametophyte, embryo, and seed in *Senna corymbosa* (Lam.) H.S. Irwin & Barneby (Leguminosae – Caesalpinioideae). *Bot. J. Linn. Soc.*, 153(2): 169-179.

Rodriguez-Pontes, M. 2008. Seed formation in two species of *Adesmia* (Fabaceae): co-occurrence of micropylar and lateral endosperm haustoria in legumes and its taxonomic value. *Bot. J. Linn. Soc.*, 158(4): 602-612.

Shinwari, Z.K., K. Jamil and N. B. Zahra. 2014. Molecular Systematics of Selected Genera of Family Fabaceae. *Pak. J. Bot.*, 46(2): 591-598.

Sutton, R.F. and R.W. Tinus. 1983. Root and root system terminology. *For. Sci.*, 24(4): 138.

Takhtajan, A. 2009. *Flowering Plants*. (2nd Ed) Springer, New York.

Wang, X.Y., Y.F. Li and J.H. Shen. 1991. Studies on the embryology of *Glycine gracilis*. *Natural Sci. J. Harbin Nor. Uni.*, 7(1): 61-71.

Zhang, S.L., Y.F. Zhang and J.H. Shen. 1990. Investigation on embryology of wild soybean. *Natural Sci. J. Harbin Nor. Uni.*, 6(3): 82-90.

Zhang, X.X., M. Liu, M.Y. Wang, C.Q. Shi and X.Y. Cheng. 2013. Developmental and morphological study of the coleorhizae in *Hemerocallis* (Liliaceae). *Pak. J. Bot.*, 45(5): 1673-1676.

Zhang, Y., S.F. Li, Q. Wang, Y. Li and R.B. Zhang. 2010. Study on seed germination characters of *Mimosa pudica* L. *Chin. Agr. Sci. Bull.*, 26(24): 52-55.