A CONTRIBUTION TO THE EMBRYOLOGY OF *RHYNCHELYTRUM REPENS* (WILLD) C.E. HUBBARD

Mohammed Inamuddin*, **Beatrice Were**** and **Mohammad Saquib***
*Department of Biology, Faculty of Natural Sciences, University of Guyana.
**Deprtment of Botany,Chepkoilel Campus,Moi University,Eldoret,Kenya.
***Department of Biological Sciences, Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria

Abstract: The present investigation deals with morphological and embryological studies of *Rhynchelytrum repens* (Willd) C.E. Hubbard. The development of anther walls are found to be Monocotyledonous type. The tapetal cells are substantially large, glandular and uninucleate. The middle layer is ephemeral and their cells are small in size. It is sandwiched between endothecial and tapetal layer. The endothecial cells are large and develop fibrous thickenings. The microspore mother cell undergoes two successive reduction divisions, giving rise to isobilateral microspore tetrad. The tetrad separates and give rise to four pollen grains. Occasionally, the anther show degenerating pollen grains before dehiscence. Formation of Ubisch's bodies has also been observed. The pollen grains shed at three celled stage. The exine is thick while intine is thin.

The ovule is anatropous, bitegmic and crassinucellate. The female archesporial cell becomes large with dense cytoplasm. It directly functions as megaspore mother cell and undergoes two meiotic divisions to produce a linear megaspore tetrad. The micropylar three cells degenerate and chalazal one becomes functional. The chalazal functional megaspore undergoes three mitotic divisions without wall formation and produces 8-nucleate embryosac. Such 8-nucleate embryosac organizes into Polygonum type of embryosac. It is interesting to note that some somatic cells of the ovule undergo nuclear divisions and give rise to facultative apomictic embryosacs.

Key Words: Eldoret; Microsporangium; Ubisch's bodies; Facultative apomixis; Female gametophyte.

INTRODUCTION

Poaceae is a large family of wide distribution comprising divergent and polymorphic group of plants. The family has attracted the attention of botanists for a long time due to its interesting morphological, anatomical, cytological and embryological features besides its economic importance. The genus *Rhynchelytrum* belongs to the tribe Paniceae of the sub-family Panicoideae and consists of over 15-species, mostly restricted to Africa, four of which are confined to Kenya (Ibrahim and Kabuye,1987). In East Africa it usually grows as weed of disturbed habitat of old farmland (Bogdan, 1975). This species plays an important role in the fixation of gaseous nitrogen in the soil (Skerman and Reveros, 1990). However, no embryological studies has been done so far. Therefore, it was considered desirable to undertake *Rhynchelytrum repens* to study its embryology.

MATERIAL AND METHODS

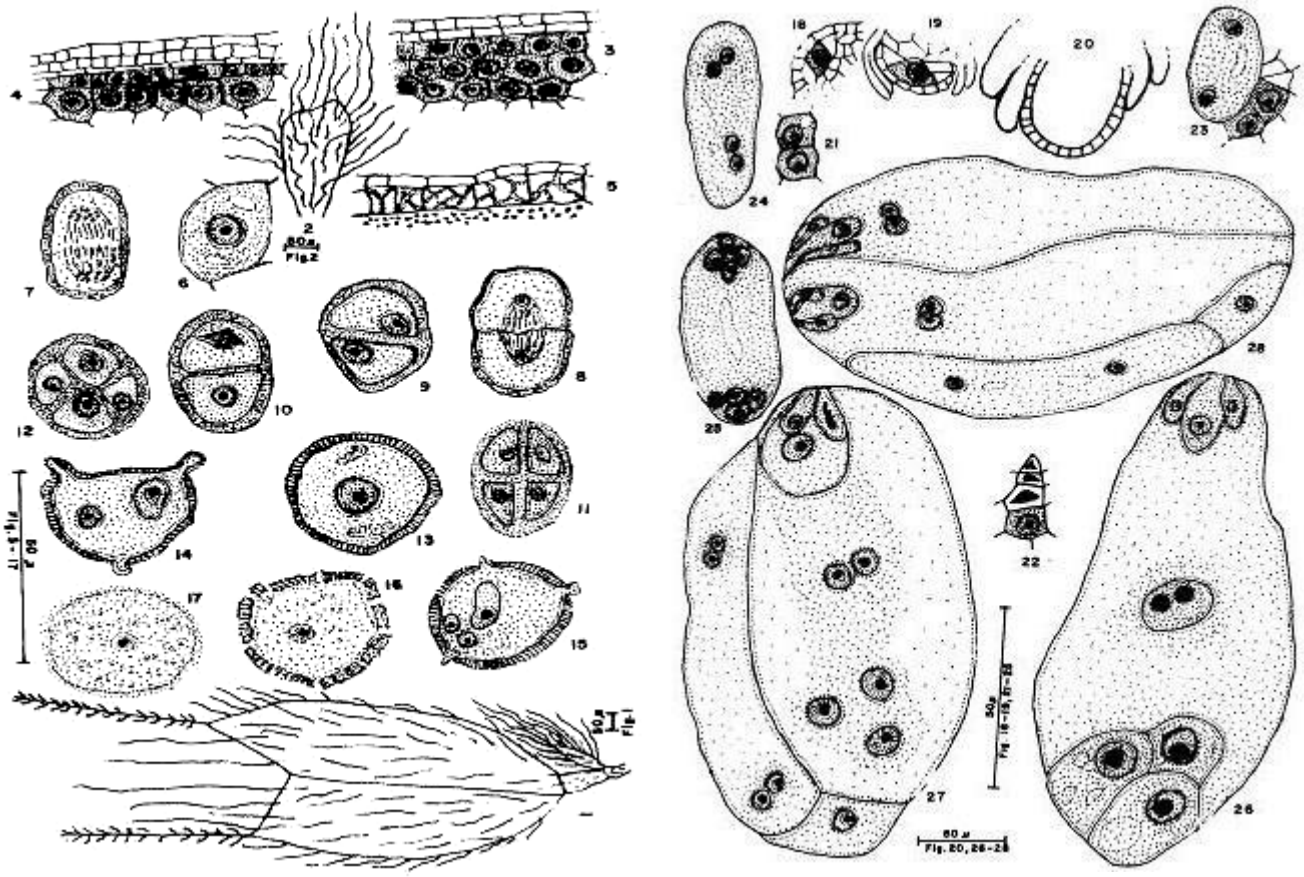
The plants were collected from college campus of Moi University, Chepkoilel, Eldoret. Some plants were pressed for morphological studies while flower buds at different stages of development were collected and fixed simultaneously in 1:3 acetic alcohol for embryological studies. The herbarium specimens were deposited to Botany department, Moi University for future reference. The dissected flower buds were dehydrated in alcohol-xylol series. The dehydrated materials were embedded in paraffin wax and microtome sections were cut at 5-9 microns thickness. The sections were stained with safranin and fast green combination.

RESULTS

MOPRPHOLOGY

Rhynchelytrum repens is a perennial grass with erect culms ranging from 25-125 cm in height. Sometimes, it is densely tufted with an accumulation of leaves at the base. Rarely the tufted plants bear stolons. The leaves may be with and without hairs. Leaf sheath is glabrous or tomentose. Leaf blade is about 6.0-13.5 cm long .The spikelets are wooly, ovate, silvery, pink or purple in colour. It measures about 40-60 mm in length. It is noteworthy that at the base of each spikelet, a

Author for Correspondence: Mohammad Saquib, Department of Biological Sciences, Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria. E-mail: mohammadaqib@hotmail.com.



Figs. 1-28: *Rhynchelytrum repens.*

Fig.1: Showing spikelet. **Fig.2:** Showing appendage with a notch at apex. **Fig.3:** Showing parietal layer and sporogenous tissue. **Fig.4:** Anther showing wall layers and microspore mother cells. **Fig.5:** Showing endothecium with fibrous thickenings and Ubisch's bodies. **Fig.6:** Microspore mother cell before cell division. **Figs.7-8:** Microspore mother cell in meiosis I. **Fig.9:** Showing dyad. **Fig.10:** Showing second meiotic division. **Fig.11:** Showing isobilateral microspore tetrad. **Fig.12:** Decussate microspore tetrad. **Fig.13:** Showing one celled pollen grain. **Fig.14:** Showing two celled pollen grain. **Fig.15:** Showing three celled pollen grain. **Figs.16-17:** Showing degeneration of pollen grains. **Fig.18:** L.s.ovule showing archesporial cell. **Fig.19:** L.s.ovule showing megaspore mother cell and integuments. **Fig.20:** L.s.ovule showing anatropous condition. **Fig.21:** showing dyad. **Fig.22:** showing megaspore tetrad in which three micropylar megaspores are degenerating and chalazal one is functional. **Fig.23:** Showing 2-nucleate embryosac and two healthy somatic cells. **Fig.24:** Showing 4-nucleate embryosac. **Fig.25:** Showing 8-nucleate embryosac. **Fig.26:** Showing mature embryosac. **Fig.27:** Showing one sexual and two apomictic embryosacs. **Fig.28:** Showing two multiple embryosacs and two apomictic embryosacs.

short leaf like appendage is present which consists of notch at the apex(Figs.1-2). Its, size, structure and position is constant in both tufted as well as erect type of plants. The appendage is very hairy and the hairs extend beyond its entire length. The upper and lower glumes consist of awns and they are not always straight. The awns measure about 5-35 mm in length. They are generally curved on the back side .The palea is boat- shaped and the lodicules are hairy. Each spikelet consists of an upper perfect floret and a lower staminate floret. The perfect floret comprises of three stamens with versatile anthers and a feathery bifid stigma while the staminate floret bears only three anthers .The ovary is superior with one ovule in each locul with basal placentation. The ovule is anatropous and produces a caryopsis on maturity.

MICRSPORANGIUM

The young anther of *R. repens* is composed of a homogenous mass of parechymatous cells, enclosed by an epidermis. The cells in the central region of the anther are smaller than the other cells which are future vascular trace of the connective. The hypodermal archesporial cell start differentiating very early at the four corners of each anther. The cells can be distinguished by the presence of dense cytoplasm and more prominent nuclei. Each archesporial cell divides periclinally to give rise to an inner and outer primary parietal layers. The inner primary parietal layer cells undergo mitotic divisions in various planes to give rise to sporogenous tissue towards the center of the anther(Fig.3). The outer primary parietal layer cells further divide periclinally to give rise to anther walls conforming to Monocotyledonous type(Davis,1966).The mature anther walls consists of epidermis, endothecium, middle layer and a tapetal layer(Fig 4). At maturity the endothecial layer cells acquire fibrous thickenings(Fig.5).The middle layer is sandwiched between the enlarged endothecial and tapetal cells which later on degenerate and disappear. The tapetum is single layered, glandular and uninucleate. Sometimes, at places the cells become 2-nucleate. The tapetal cells begin to collapse at 1-celled stage of pollen grains. It is interesting to note

that the inner wall of the tapetum, after the formation of pollen grains, breaks and forms small spherical and glandular bodies known as Ubisch's bodies(Fig.5). These bodies remain adpressed to the inner wall of endothecium.

MICROSPOROGENESIS

The microspore mother cells undergo first meiotic division resulting in the formation of dyads(Figs.6-10).The dyads undergo further division to form isobilateral microspore tetrads(Fig.11). The microspore tetrads are normally produced by successive divisions. Rarely, decussate microspore tetrads have also been observed (Fig.12).

MALE GAMETOPHYTE

The young microspores are liberated in the anther locule and become rounded(Fig.13). The exine is moderately thick and opaque while the intine is thin. The mature pollen grains lack starch grains.

It is important to note that sometimes, the pollen grain wall bursts which later on degenerates and disappears(Figs.16-17). The nucleus of the normal pollen grain undergoes mitotic division resulting in the formation of large vegetative and a small generative nucleus(Fig.14). The generative nucleus later divides into two to form male gametes(Fig.15). Thus, the mature pollen grains are shed at 3-celled stage.

MEGASPORANGIUM

Some of the hypodermal cells of young placenta undergo cell divisions in various planes resulting in the formation of dome-shaped ovule primordia. The nucellus is represented by a layer of epidermal cells (Figs.18-20). The two integuments develop from the epidermal and sub-epidermal layers of the ovule soon after the differentiation of the archesporium. The inner integument appears first and later it is followed by the outer integument. During development, the ovule undergoes a gradual change of curvature until it becomes anatropous (Fig.20). The micropyle is formed by the inner integument. The inner integument is less massive than the outer one.

MEGASPOROGENESIS

Prior to the initiation of the integuments a single hypodermal cell, immediately behind the nucellus of the ovule becomes large and densely cytoplasmic with a prominent nucleus differentiating as an archesporium (Fig.18). The archesporial cell directly functions as megaspore mother cell (Fig.19). The megaspore mother cell undergoes meiosis first and gives rise to a dyad (Fig.21). The second division of the dyad results in the formation of a linear megaspore tetrad (Fig.22). The upper three megaspores degenerate while the chalazal one becomes functional.

FEMALE GAMETOPHYTE

The functional megaspore becomes prominent and attains a significant size. The centrally located nucleus divides mitotically into two and the resulting nuclei move to opposite poles forming 2-nucleate embryosac (Fig.23). The two nucleus of the poles undergo further division resulting in the formation of a 4-nucleate embryosac (Fig.24). The third nuclear division produces 8-nucleate embryosac and gradually the sac increases in size (Fig.25). The micropylar quartet nuclei give rise to an egg apparatus at the extreme of the sac which consists of an egg cell and two synergids. The fourth nucleus of the micropylar region move to the centre of the embryosac to form a polar nucleus. The three nuclei of the chalazal quartet form three antipodal cells at the chalazal end of the embryosac and the fourth nucleus moves towards the centre of the sac and functions as lower polar nucleus. The two polar nuclei fuse to form a secondary nucleus prior to the entry of pollen tube into the sac. The development of the embryosac thus conforms to the Polygonum type (Fig.26). Occasionally in mature embryosac the antipodal nuclei do not differentiate into antipodal cells. They tend to remain free in the sac (Fig.27).

Besides the embryosac formed from the megaspore, there is evidence that other somatic cells of the ovule become potentially active and assume the function of megaspores. They undergo mitotic divisions to form apomictic embryosacs. In an ovule of 2-nucleate sexual embryosac, some of the somatic cells near the sac appear to act as megaspores (Fig.23). In another ovule, 3-embryosacs are observed, one of which is 8-nucleate sexual embryosac where the antipodal nuclei are yet to organise and the other two, 4-nucleate and uninucleate embryosacs are apomictic (Fig.27).Still in another ovule, there are two multiple sexual embryosacs without antipodal cells while adjacent to it there are two apomictic embryosacs (Fig.28). Among them one of the embryosacs is 2-nucleate and the other is uninucleate. The position of the multiple sexual embryosacs and their simultaneous development suggest that they could have developed from two megaspores of a tetrad or from two separate functional megaspores.

DISCUSSION

The grass flora of Kenya is very rich comprising about 606 species (Ibrahim and Kabuye, 1987). The different species of the genus *Rhynchelytrum* are mostly found in Africa. Bogdan (1958) reported six species of this genus while Ibrahim and Kabuye (1987) on the basis of their own key, reported only four species. However, the species *R. repens* shows a great range of morphological variation with regard to its indentification. Such variation of characters include leaf blade length and breadth, presence or absence of hairs on leaf as well as tufted or not tufted nature of the plant. These variations suggest that the present taxon needs further more investigation. It is worth to note that the presence of leaf-like appendage at the base of each spikelet may be used as a useful character in identification of different species of the genus which was not reported earlier. The rare presence of stolon in tufted plants suggests that they have the power of adaptation of environment.

The present work on the embryology of *R. repens* is the first investigation of the genus. The occurrence of tetrasporangiate anther and the development of Monocotyledonous type of anther walls in the present material is essentially similar to other investigated members of the family Poaceae (Davis,1966). Contrary to Davis (1966) the tapetal cells in the described species are 2-nucleate except in *Pennisetum*

typhoideum, where they are uninucleate. However, the presence of uninucleate tapetal cells in *Pennisetum divisum* and *Pennisetum setaceum* (Inamuddin and Faruqi, 1982), *Panicum turgidum* (Inamuddin and Abdulla,1990), *Stipagrostis ciliata* (Inamuddin and Maghboub, 1990) and in *R.repens* is an interesting feature.

Successive cytokinesis in microspore mother cells including *R.repens*, is the prevalent character of the poaceae, although simultaneous type of cytokinesis has also been reported in *Soghum vulgare* (Artschwager and McGuire, 1949).

The presence of Ubisch's bodies in the anther of *R. repens* is worth mentioning. Although such structures have been reported earlier in some other species of the family (Bhojwani and Bhatnagar, 1983). According to them the Ubisch's bodies are useful in the external thickening of the exine of pollen grains. The formation of isobilateral microspore tetrad in *R. repens* is in conformity with other investigated members of the family (Davis, 1966). The rare occurrence of decussate microspore tetrads in the present material is not a unique feature. It has been recorded earlier in *Triticum diccocum* (Deshpande and Raju, 1985) and *Stipagrostis ciliata* (Inamuddin and Maghboub,1990). However, according to the former, the formation of decussate microspore tetrads is nothing but a modification of isobilateral microspore tetrad. The occasional degeneration of pollen grains of *R .repens* is an important feature. A similar phenomenon has been reported earlier in *Sorgham bicolor* (Penchoksarappa and Annigeri, 1984; Narayana et al., 1985) *Arnebia hispidissima* (Baquar and Hussain, 1969). According to the former authors, on the basis of histochemical studies, the sterility and degeneration of pollen grains could be due to carbohydrate deficiency in tapetal cells while the latter authors are of the opinion that such sterlity may result from cytomixis. However, in the present material, no cytomixis was observed and therefore, pollen grain degeneration may be attributed to carbohydrate deficiency or failure of anther dehiscence.

The development of Polygonum type of embryo sac in *R. repens* is essentially similar to the other investigated members of the family (Davis, 1966). The occurrence of multiple embryosacs in the present material is common as observed in the other grasses (Davis, 1966).

The development of embryosac from the somatic cells of the ovule adjacent to the sexual embryo sac without tetrad formation, provide a strong evidence that apomixis is present in *R. repens*. According to Brown and Emery (1958) the sexual embryo sac in panicoid grasses are 8-nucleate while the apomictic embryosac is 4-nucleate. The results of the present study confirm to his observations. However, 8 nucleate somatic embryosac has been considered as apomictic in *Pennisetum dubium* (Gildenhys and Briz, 1959). Since both sexual and apomictic embryo sacs are formed in the ovule of *R. repens*, suggest that the apomixis is of facultative type (Carnahan and Helen, 1961).

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