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Apomixis in *Genista salzmannii* DC. (Fabaceae)

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ABSTRACT. — *Genista* apomixis is described for the first time in two populations of *Genista salzmannii* DC.: the first is diploid and the second one tetraploid. There was no female meiosis and the megaspore mother cell functioned as an unreduced megaspore, which led to the mature *Antennaria* type embryo sac. A hybrid origin hypothesis for the species was made.

Key words: embryology, apomixis, *Genista salzmannii* DC.

INTRODUCTION

The *Genista* genus, which consists of about 80 taxa distributed for the most part in the Mediterranean area, was divided by SPACH (1844) and then by GIBBS (1966) into various subgenera and sectiones. The sectio *Erinacoides* Spach, integrated recently with other species (VALSECCHI, 1984; 1986), corresponds almost totally with the aggregatum of *Genista aspalathoides* set out in the MED-CHECKLIST (GREUTER *et al.*, 1989). From a systematic view-point, this sectio is one of the most complex: it includes closely related endemic taxa present in mountainous areas often quite close together.

Subgenere *Genista* (Spach) Gibbs
Sez. *Erinacoides* Spach

<i>Genista aspalathoides</i> Lam.	Sicily, Algeria, Tunisia
<i>Genista parnassica</i> Halácsy	Greece, Aegean Isles
<i>Genista lobelii</i> DC.	France, Spain, Corsica, Algeria, Marocco
<i>Genista lobelii</i> DC. ssp. <i>longipes</i> (Pau) Heyw.	Spain
<i>Genista hystrix</i> Lange	Spain, Portugal
<i>Genista legionensis</i> (Pau) Lainz	Spain
<i>Genista mugronensis</i> Vierh. ssp. <i>mugronensis</i>	Spain
<i>Genista mugronensis</i> Vierh. ssp. <i>rigidissima</i> (Vierh.) Fernand. Cas.	Spain
<i>Genista polyanthos</i> Willk.	Spain, Portugal
<i>Genista sanabrensis</i> Valdés	Spain

<i>Genista versicolor</i> Boiss. ssp. <i>versicolor</i>	Spain
<i>Genista versicolor</i> Boiss. ssp. <i>pumila</i> (Hervier) Fernand. Cas.	Spain
<i>Genista arbusensis</i> Valsecchi	Sardinia
<i>Genista desoleana</i> Valsecchi	Sardinia, Corsica, Liguria, Elba
<i>Genista salzmannii</i> DC.	Sardinia, Corsica
<i>Genista sulcitana</i> Valsecchi	Sardinia
<i>Genista toluensis</i> Valsecchi	Sardinia

This particular distribution, which permits the identification of secondary centres of differentiation (e. g. the Iberian Peninsula and the Sardinian-Corsican complex), presupposes a relatively recent speciation in this *Genista* group. This led us to investigate the reproduction of one of them, *Genista salzmannii* DC., so as to identify any possible anomaly in the reproductive phase which could have influenced this particular differentiation.

There have been very few studies on the reproduction of the *Genista* genus or other similar ones (CORTI, 1930, 1946, 1949, 1950; SOUÈGES, 1947; MOGGI, 1950; GRAMUGLIO & ROSSO, 1968); these, for the most part, are about embryological and embryogenetic development of few taxa and all relate a normal development and amphimixis.

MATERIALS AND METHODS

Material for this study was taken from two Sardinian populations of *Genista salzmannii*: one diploid ($2n = 18$, VILLA, 1988) present in the Marghine chain of mountains in Ortachis; the other tetraploid ($2n = 36$, VILLA, 1988) from Mount Limbara. Buds and flowers were collected at different stages of development, fixed in ethyl alcohol-glacial acetic acid (5:2; Carnoy), embedded in paraffin, sectioned at 14μ and 16μ and stained with Delafield's hematoxylin. There were notable difficulties in orienting the cut of the ovaries, thus it was decided to attempt observation after clarification. The florets, fixed in Carnoy, were first stained with Mayer's haemalum and subsequently clarified with methyl-salicylate (STELLY *et al.*, 1984). Single ovules and anthers were then isolated and studied by optic microscope. The results obtained were satisfactory only for microsporogenesis and for the first differentiation stages of the ovules, since, as the integument thickened it was not possible to observe the gametophyte in more advanced stages. The whole ovaries thus treated were at this point embedded in paraffin and sectioned normally (orientation was significantly easier). Over 500 flowers for a total of approximately 2,000 ovules were studied.

RESULTS

Ovule development

The description of the various stages of development in the ovule regards the tetraploid *Genista salzmannii* population from Limbara. At the end of this paragraph, differences, if any, found in the diploid population from the Marghine are listed.

The ovules, 4 or 5 per ovary, begin to differentiate themselves from the placenta, increase and the funiculus enlarges and becomes extended. At this stage their orienta-

tion is orthotropic, they measure 150-180 μ , and the integuments are sketchy. In the nucellus, which consists of a cell mass (crassinucellate ovule), a hypodermal cell initiates differentiation into an archesporial cell which then cuts off into a primary parietal cell and a megaspore mother cell. The parietal cell, by successive divisions, forms the parietal tissue. The megaspore mother cell (Figs. 1, 12) increases until it becomes approximately five times larger (about 40 μ long) than the surrounding cells (Figs. 2, 13). The archesporium proved to be, in one or two cases, bicellular, with two mother cells of more or less similar dimension one above the other (Figs. 3, 15); it is, however, always the upper cell which develops.

The thus formed mother cell does not enter into meiosis but, after a long interphase growth, its nucleus moves towards the micropylar area, whereas, at the chalazal pole, a spherical mass of denser granulations may be seen in the cytoplasm (Figs. 4, 14). Such a formation had already been noted (CORTI, 1950) in *Cytisus canariensis* and explained as being an accumulation of non-identified reserve substances localized in the cellular pole, which becomes, after meiosis, the only spore to germinate.

Contemporaneously to this phase there is an increase in the ovule: the integuments, both formed of two cell layers, thicken but do not yet form the micropyle. The ovule briefly remains orthotropic but, shortly after, starts to rotate towards the upper part of the ovary until finally it arrives at the characteristic campilotropic position with a zig-zag micropyle, that is, with a greater increase in one part of the external integument. This part of the external integument, facing the ovarian cavity, in addition to the above mentioned increase, also becomes thicker in the marginal zone; this is caused by the increase of the cell layers which go to form the aryl. Despite the high number of ovules seen at megaspore mother cell stage, meiotic initial phases were not observed.

The mother cell starts to vacuolize and produces directly by gonial apospory (BATTAGLIA, 1963) or mitotic diplospory (NOGLER, 1984) an unreduced gametophyte of the *Antennaria* type.

The mononucleate begins to enlarge and the first vacuole appears in the cytoplasm near the micropyle above the nucleus (Figs. 5, 16). This then divides giving rise to a binucleate apocytium (Fig. 6). The four-nucleate stage (Figs. 7, 8, 17) may come about with both parallel and crossed spindles, as may be deduced from the position of the nuclei since mitotic phases were never observed (these probably take place at night). Four-nucleate division leads to the formation of an 8-nucleate. Three nuclei quickly form a typical egg apparatus of two synergids, which cellularize before the other nuclei, and the egg cell (Fig. 9). Synergids are pear-shaped, with apex towards the micropyle and base towards the egg cell which is very large and becomes a cuplike shaped (Figs. 18, 19). The two polar nuclei are very close together and are clearly evident towards the centre of the sac, not very far from the egg cell. Cellularization in the antipodal apparatus was not clearly observed: usually a single, very small nucleus is present in addition to the remains of two or three degenerated nuclei.

In only one ovule it was possible to observe a binucleate with obvious remains of three degenerated spores (Figs. 11, 21). This confirms the fact that meiosis in *G. salzmännii* is an exceptional event; further confirmation of this was that in the other ovules from the same ovary, which had probably developed apomictically, the gametophytes present were 8-nucleate.

Additional differentiations occur in the nucellus at this stage: chalazal zone cells elongate and become finer, their cytoplasm attaches itself to embryosac-walls, and the nuclei move to one side and seem very small and quiescent. These cells make up a true chalazal apparatus with probable trophic functions. The two inner cell-layers of the nucellus differentiate so as to form an internal lining or endothelium, with thicker-walled cells similar to those described in other Fabaceae (DAVIS, 1966).

Fecundation does not occur; but rather, the egg cell, by means of direct diploid parthenogenesis, gives rise to a proembryo which was observed only in the first stages of division (fecundation by porogamy observed by Corti and Moggi in other similar species was not seen in *G. salzmannii*).

The two fused unreduced polar nuclei form an autonomous endosperm, consisting in a very lax apocytium with numerous nuclei, often with more than one nucleolus, which fill the entire cavity of the sac up to the chalazal pole. A large part of the ovules reaches maturity and forms fertile seeds.

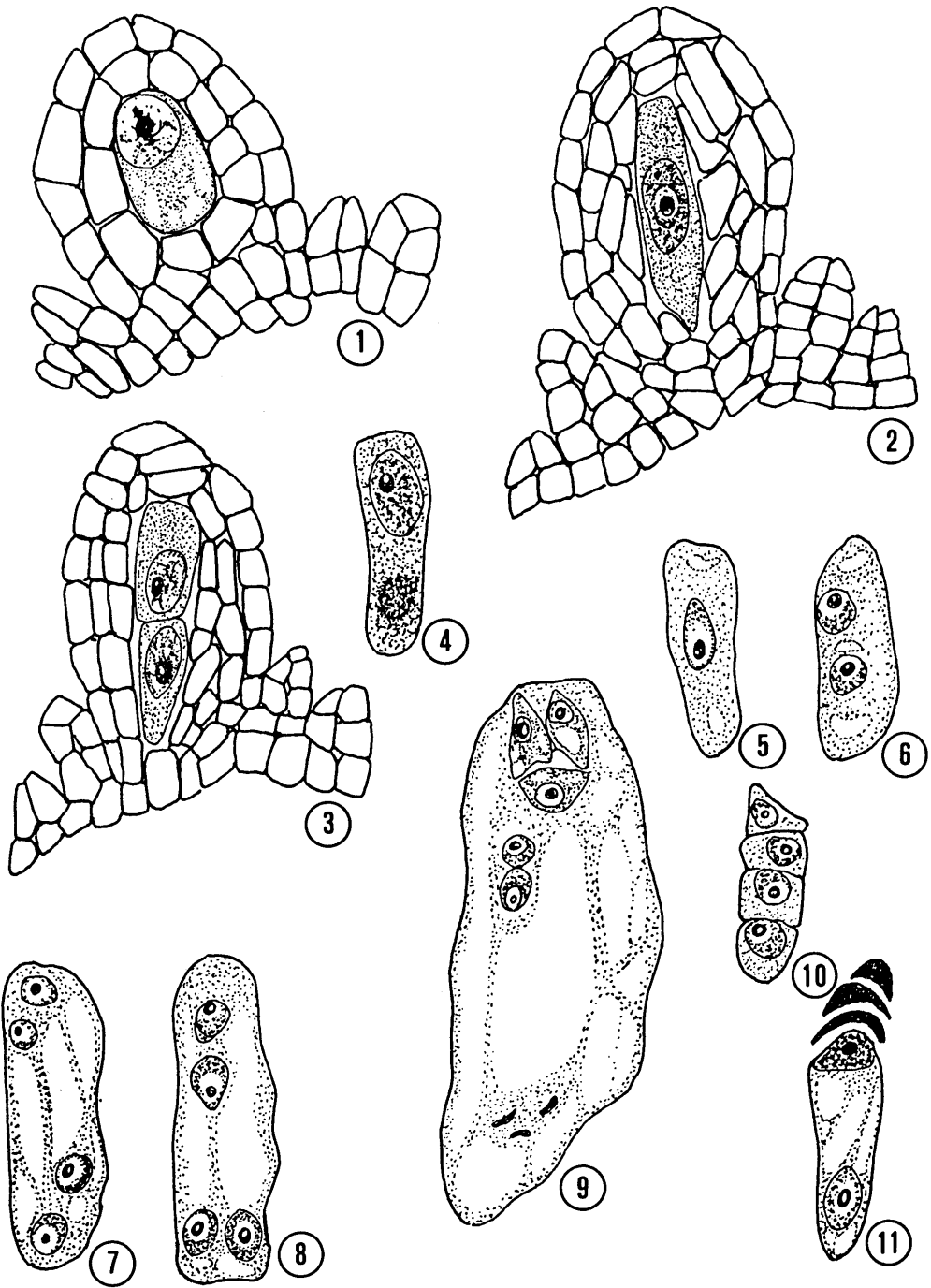
The development cycle of the diploid population of *G. salzmannii* from the Marghine also proved to be apomictic. Particular observations were:

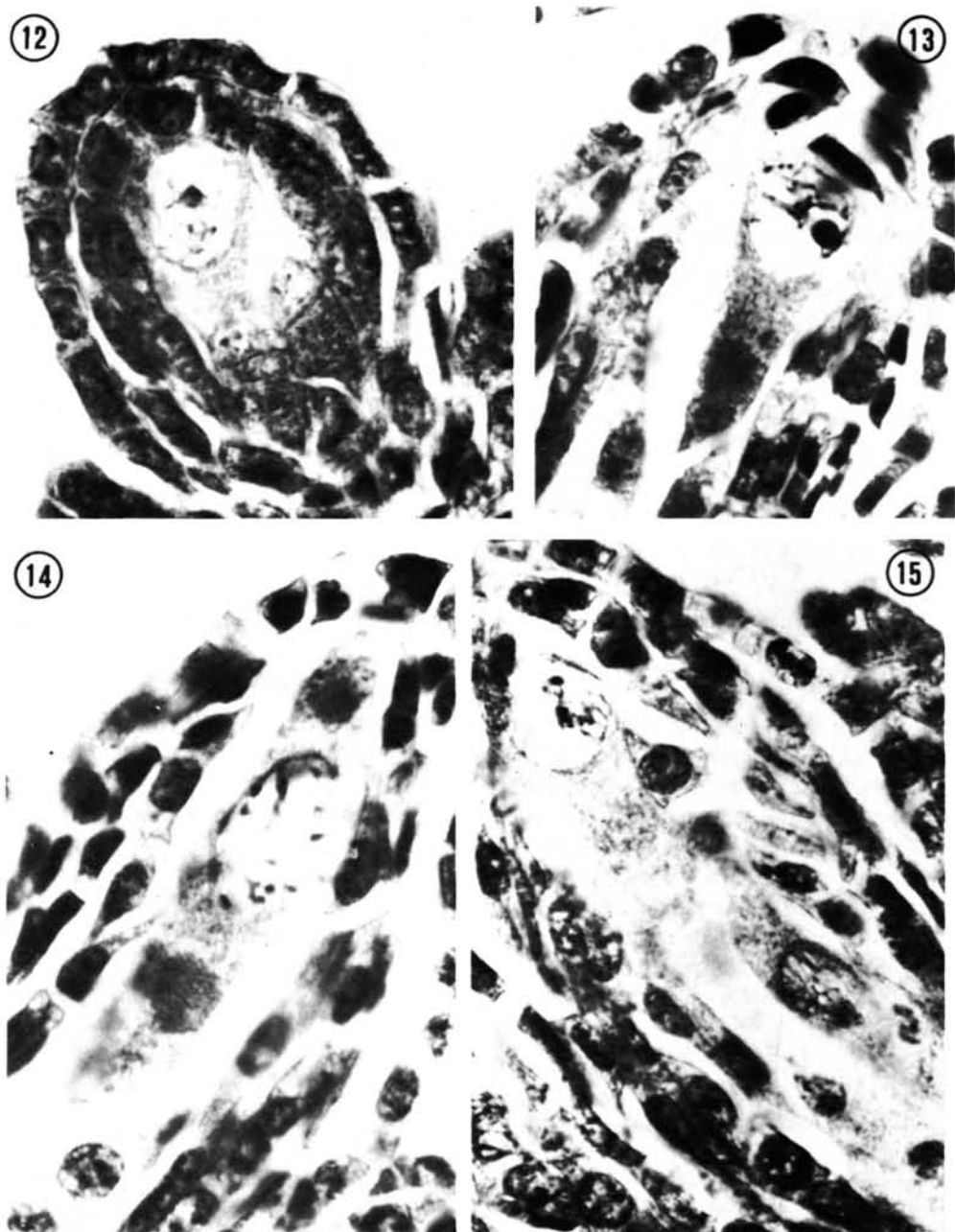
- the presence, observed in a single ovule, of a megaspore tetrad (Figs. 10, 20) while the other ovules from the same ovary showed quiescent mother cells suggests that in this population meiosis is likewise an exceptional event;
- the mature ovule is of smaller size;
- the seeds that reach maturation are very few or, in some plants, totally absent.

Microsporogenesis

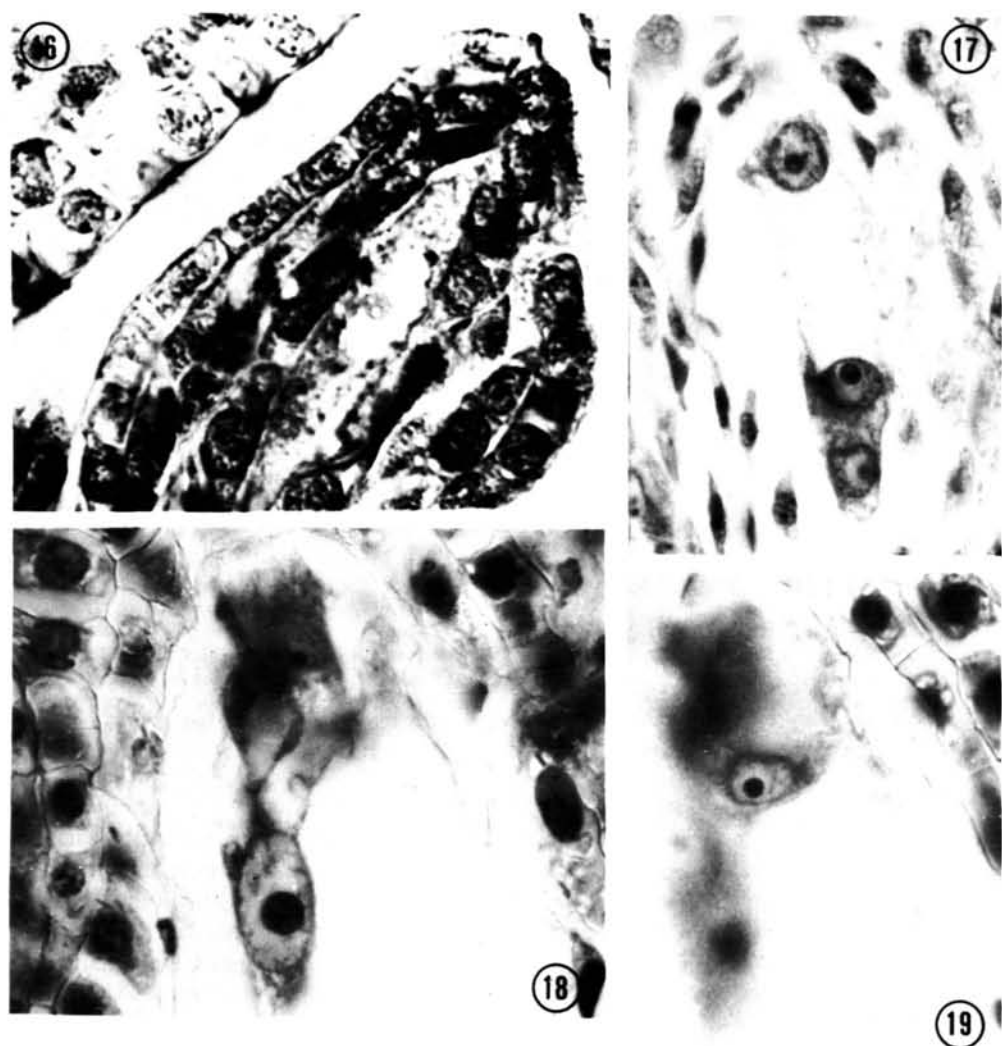
The formation of pollen follows in this species usual sequence: it begins with mother cell differentiation from the archesporial tissue, along with the contemporaneous formation of macrspore mother cells in the ovule. The latter remains quiescent for a long period, whereas the meiotic phases of microsporogenesis occur quite quickly, thus leading via simultaneous division to tetrad formation and then to mature binucleate pollen granules. In both Limbara and Marghine specimens of *G. salzmannii*, meiosis takes place in the same way: the only difference observed is that it proceeds with greater regularity in the tetraploid population, while in the diploid the quantity of granules reaching maturity is much less. Indeed, up until the tetraspore stage, divisions proceed in an apparently normal way; subsequently, however, many degenerations are observed in the diploid so that, at full maturation, the anthers contain very few pollen grains.

Figs 1 - 11 — Ovules longitudinal sections at successive stages of its development. (ca. x 700).
1 - Megaspore mother cell (MMC) in a young ovule. 2 - Increased MMC. 3 - Two MMCs in the same ovule. 4 - MMC with a spherical mass of denser granulations towards the lower pole. 5 - Unreduced mononucleate embryo sac. 6 - Unreduced binucleate embryo sac. 7, 8 - Unreduced tetranucleate embryo sac. 9 - Unreduced female gametophyte. 10 - The only tetrad observed. 11 - The only binucleate embryo sac observed with three degenerated spores.





Figs. 12 - 15 — Ovules longitudinal sections with MMC at successive stages (ca. x 880).
15 - Two MMCs in the same ovule.



Figs. 16 - 19 — Gametophyte development (ca. x 880).
 16 - Unreduced mononucleate embryo sac. 17 - Unreduced tetranucleate embryo sac.
 18, 19 - Egg apparatus and polar nucleus in two successive sections.

CONCLUSIONS

Apomictic phenomena in the *Genista* genus are here shown for the first time. In fact, in the study of two populations of *G. salzmannii*, one diploid and the other tetraploid, it was observed that the embryo sac originated by means of mitotic diplospory of the *Antennaria* type (NOGLER, 1984). This phenomenon was practically generalized in both populations. However, one case of tetrad formation was observed in one population, whereas a case of binucleate embryo sac and remains of three degenerate macrospores

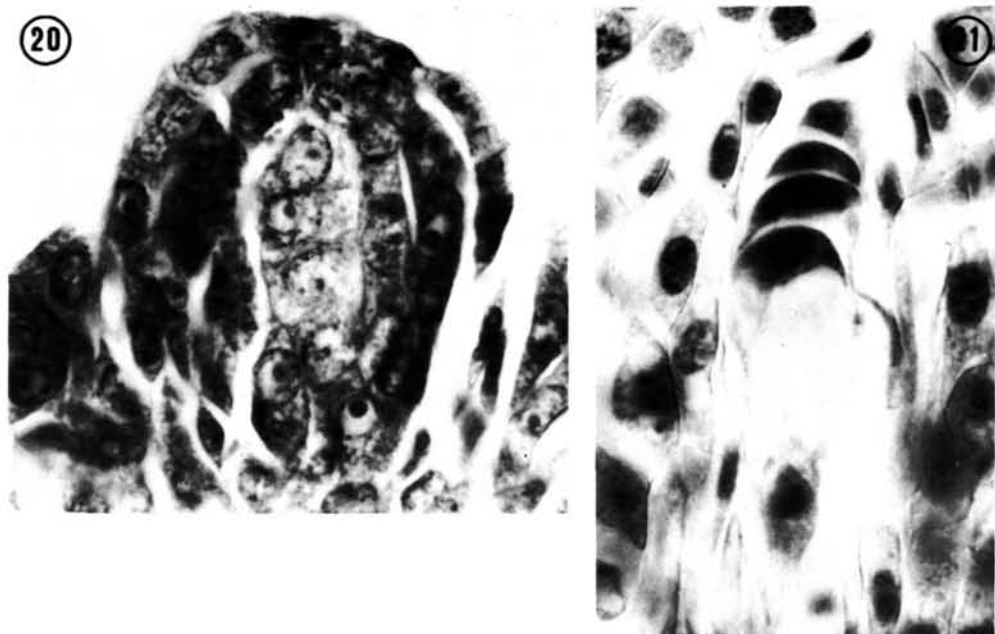


Fig. 20 — The only tetrad observed (ca. x 880).

Fig. 21 — The only binucleate embryo sac observed with three degenerated spores (ca. x 880).

were found in the other. Such cases, therefore, prove that sexuality is not completely suppressed; however, no presence of pollen tubes in the ovary nor cases of fertilization were observed. Microsporogenesis, which seems to proceed normally in the first phases, produces abundant pollen granules in the tetraploid species, but very little in the diploid species on account of degenerative phenomena after tetraspore formation. Production of fertile seeds is also very high in the tetraploid population, but low in some specimens of the diploid population.

The good pollen set in the 4x biotype and the poor set in the 2x biotype suggest that *G. salzmannii* could be composed by diploid hybrids and allotetraploids. Diploids could have originated tetraploids, but more likely the opposite could have happened so that tetraploids would be ancestral to diploids. According to HARLAN & DE WET (1975) the great majority of tetraploids have originated by fusion of diploid unreduced gametes without passing through the diploid-hybrid stage. The 2x biotype could indeed have arisen from a rare reduced egg of a 4x individual that has developed parthenogenetically into an apomictic dihaploid. NOGLER (1984) believes that at least part of the existing diploid apomicts «has resulted from tetraploid apomicts by haploid parthenogenesis». Dihaploids are usually rare, weak, sexual and sterile, but instances are known of vigorous and apomictic ones (NOGLER, 1.c.). It would be also interesting to find out, in the future, if 2x and 4x biotypes in *G. salzmannii* are really spatially separated and if so whether diploid and tetraploid habitats are somehow different.

Apomixis is probably a latent reproductive method present in the *Genista* genus, or at least in this particular group, and could be a determining factor in the differentiation of the greater part of the taxa in the grouping of *G. aspalathoides*, which includes all the endemic species often over a restricted area.

This demonstration of apomixis in *G. salzmannii* is of particular importance, not only because it explains the speciation of the group to which it belongs, but also because it was before now practically unknown in the Fabaceae family (if we exclude one case of somatic apospory in *Trifolium pratense* L., vide HINDMARSCH, 1964).

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RIASSUNTO

Apomixis in Genista salzmannii DC. (Fabaceae). - Viene messo in evidenza per la prima volta un caso di apomixis nel genere *Genista*. Infatti dallo studio di due popolazioni, una diploide ed una tetraploide, di *G. salzmannii* è risultato che il sacco embrionale si origina per diplosporia mitotica di tipo *Antennaria*. La microsporogenesi, che decorre in modo normale, produce granuli di polline abbondanti nel biotipo tetraploide, e scarsi in quello diploide per fenomeni degenerativi dopo la formazione della tetraspora. Vengono fatte ipotesi sulla probabile origine ibridogena della specie e sulla differenziazione delle altre specie dell'aggregatum *G. aspalathoides*.