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RESEARCH NOTE

Embryo abortion and histological features in the interspecific cross between *Phaseolus vulgaris* L. and *P. coccineus* L

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Abstract In this paper, the causes of early embryo abortion in the reciprocal crosses between *Phaseolus vulgaris* L. (a cultivar) and *Phaseolus coccineus* L. (a wild form) were studied. Methacrylate resin sections, 3–5 μm thick, of 3 to 14 day-old seeds were used to examine the embryo developmental stages and the state of seed tissue. It was observed that, embryos aborted at different developmental stages (globular to early cotyledon) depending on the maternal parent. The use of *P. coccineus* cytoplasm resulted in a higher number of abortion than in reciprocal crosses. Many of them took place between 5 and 6 days after pollination (DAP). Histological analyses permitted to observe that the embryo development was slower in the cross between *P. coccineus* and *P. vulgaris*, compared to parental seeds. It would be related to a deficient endosperm development in reciprocal crosses and, in some extent, hypertrophy of the suspensor might be the main cause of early embryo abortion. Then, it would be practical to overcome this incompatibility by rescuing the embryo at the globular stage of development.

Keywords Embryogenesis · Histology · Hybridization · Incompatibility barriers · Suspensor

Abbreviations

DAP	Days after pollination
HEMA	2-Hydroxyethyl Methacrylate

Introduction

Interspecific hybridization is useful to genetically improve *Phaseolus vulgaris* genotypes for traits poorly or not expressed in the primary gene pool of common bean (Baudoin et al. 2004). But crosses within the complex *P. vulgaris – P. polyanthus – P coccineus* lead essentially to post-zygotic barriers (Geerts et al. 2002) limiting the rate of the hybridization success (Sabja et al. 1990; Debouck and Smartt 1995). The best methods to develop interspecific hybrids using *P. coccineus* as a female would be the increasing of the number of cross-pollinations and rescuing the hybrid embryos at accurate stage. To overcome post-zygotic incompatibility occurring in the wide crosses, it would be essential to study *Phaseolus* embryogenesis at the level of parental genotypes and hybrids.

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Materials and methods

A cultivar of *P. vulgaris* (NI637) and a wild form of *P. coccineus* (NI1108) genotypes from the *Phaseolineae* active collection held at the Gembloux Agricultural University were used. They were selected on the basis of their good ability to flower in growth chamber conditions. The cultivation conditions were characterized by: day/night temperature 24/20°C, relative humidity 75%, photoperiod 12h/12h and light intensity 170 μmol m^{-2} s^{-1}. Crosses were made according to Buishand's method (1956). Number of pollinations was counted and embryos abortions were computed. Seeds were collected from 3 to 14 DAP for histological study.

The histological protocol was the one, used by Lecomte et al. (1998) described by Ruzin (1999) and improved by Geerts et al. (2002). It was the histobloc operating in HEMA resin. The main steps were related to following: fixing, rinsing, dehydration, infiltration, embedding and thin serial sections of 3–5 μm in resin. Embryo length was measured and the means were computed for each one, according to its age. Data were analyzed using ANOVA and the software was Minitab vs. 14. When statistical differences were identified, a comparison of means two by two was realized using Tukey test (level of significance: 95%).

Results and discussion

Embryos aborted at different developmental stages depending on the genotype used or the cross. More than 75% of mature pods were obtained by using *P. vulgaris* (NI637) as a female. In contrast, we obtained less than 25% mature pods in the reciprocal cross (Table 1). In self-pollination, embryos abortion was low and show a slight increase from 3 to 10 DAP (Figs. 1 and 2). Many abortions occurred at 5 and 6 DAP, whatever the direction of the cross (*P. vulgaris* or *P. coccineus* as maternal parent) (Fig. 1). Important events reported by Yeung and Meinke (1993) in *Phaseolus* embryogeny were observed at this period. The suspensor was metabolically active and essential for nutrient transport. It played a major role in the growth regulators synthesis. In addition, growth of the suspensor was not inhibited by the embryo development.

Embryos of the *P. vulgaris* genotype (NI637) grew faster than the others and this was obvious from 10 DAP (Fig. 2). A significant difference ($P < 0.05$) is observed in the embryo length growth between *P. vulgaris* genotype and others. Embryos reached on average the heart stage (Figs. 3–6) 6 DAP in *P. vulgaris* (NI637), 9 DAP in *P. coccineus* (NI1108) and in the cross NI637 (♀) × NI1108, 10 DAP in the cross NI1108 (♀) × NI637. The embryo sac (sac) was separated from the embryo apical end by a thin layer of endosperm. Transfer cells (*) were arranged in series beside the endothelium (end) wall, near the embryo (emb) and the suspensor (sus) body. It developed between the endothelium and the cellular endosperm (alb) in some cases (Fig. 5). The hybrid embryo NI1108 (♀) × NI637 showed a suspensor hypertrophy (Fig. 6), resulting in smaller and delayed embryo proper, compared to selfed embryo (Figs. 3–4). At the micropylar end (mic), basal cells of the suspensor were bigger and contained larger vacuoles, compared to parental (NI637 and NI1108) and hybrid NI637 (♀) × NI1108 seeds.

In conclusion, crosses between *P. vulgaris* (♀) and *P. coccineus* were easier than reciprocal crosses. Embryos aborted like in *P. polyanthus* (♀) × *P. vulgaris* crosses (Lecomte et al. 1998). Selfed embryos aborted because of internal

Table 1 Results of the self-pollinations of *P. vulgaris* and *P. coccineus* and the crosses between the two species

Genotypes or crosses	Number of pollinations	Pods set from 3 to 14 DAP (a)	Pods harvested at maturity (b)	Rate of pods (%) reaching maturity [(b/a)*100]
NI637	121	102	96	94.1
NI1108	287	218	188	85.2
NI637(♀) × NI1108	361	271	204	75.3
NI1108(♀) × NI637	659	163	40	24.5

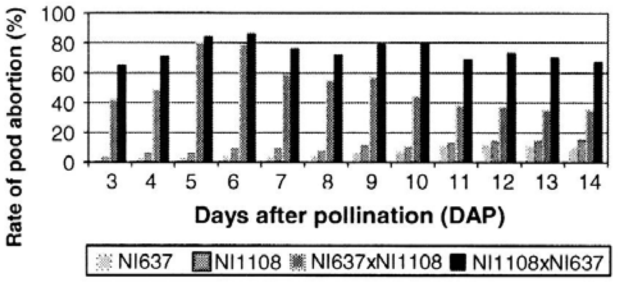

Fig. 1 Percentage of pod abortion at different days after pollination

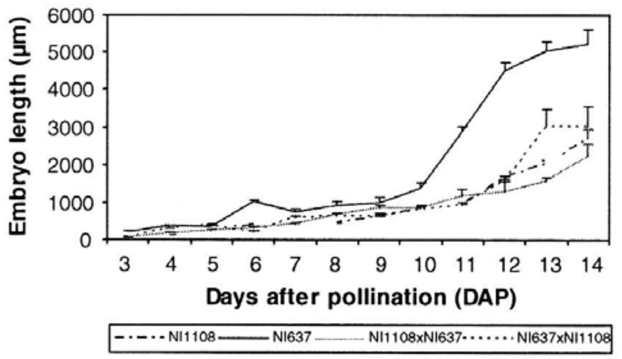

Fig. 2 Evolution of embryo length means (and standard deviation)

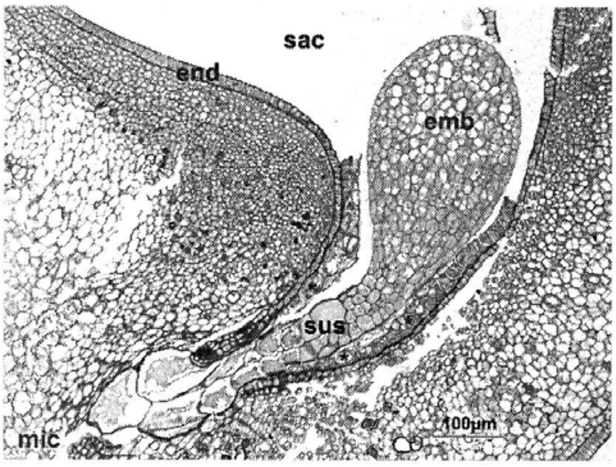

Fig. 3 A median longitudinal section of *P. vulgaris* (NI637) seed at 6 DAP showing a heart stage embryo

competition resulting from the plant phenological stage.

Developmental stages of embryos and the suspensor growth depended on the genotypes used in self-pollination and the maternal parent involved in the crosses. The suspensor was the uptake site during early stages of embryo development (Yeung 1980). In hybrid embryo NI1108

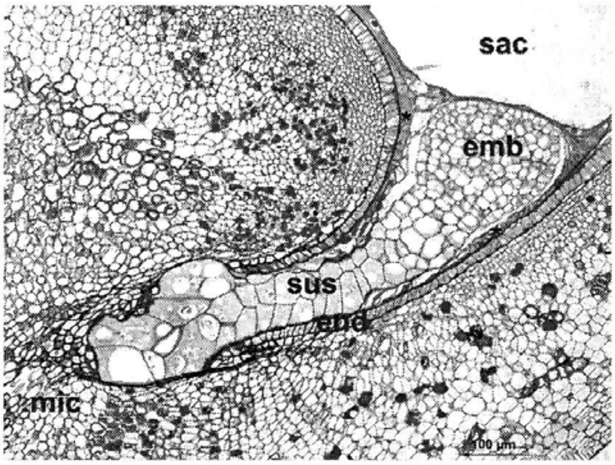

Fig. 4 A median longitudinal section of *P. coccineus* (NI1108) seed at 9 DAP showing a heart stage embryo

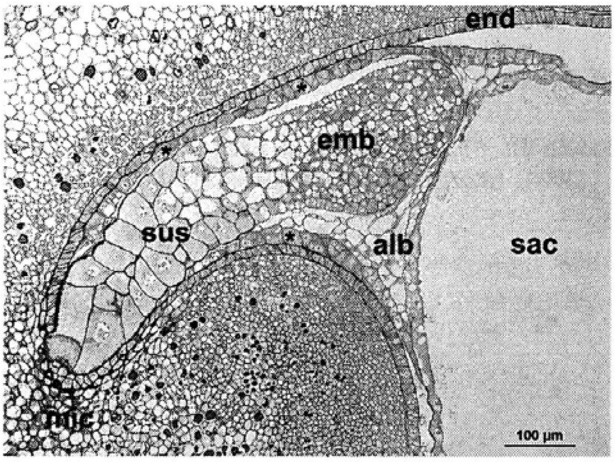

Fig. 5 A median longitudinal section in a *P. vulgaris* (♀) × *P. coccineus* seed, 9 DAP, (NI637 × NI1108) showing an embryo at heart stage

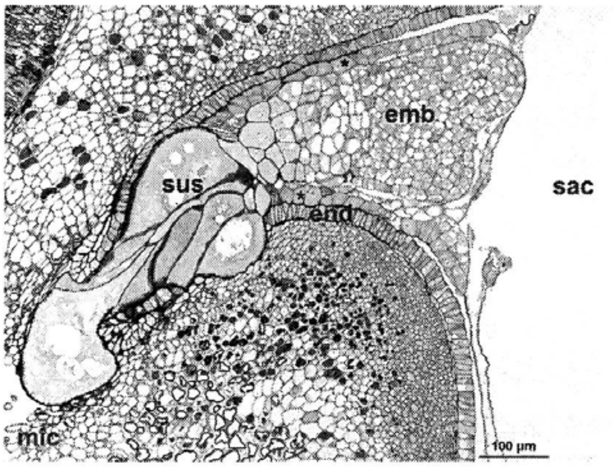

Fig. 6 A median longitudinal section in a *P. coccineus* (♀) × *P. vulgaris* seed, 10 DAP, (NI1108 × NI637) showing an embryo at heart stage with a big suspensor

(♀) × NI637 with a massive suspensor, the embryo requirement in nutrients would be greater than that of the selfed and NI637 (♀) × NI1108 hybrid embryos.

To ensure a regular embryo development and meet the nutrients requirement, it would be essential to rescue embryo for in vitro culture at the period in which critical phases of its development occurred. Observations might also be done on globular and cotyledonary stage to underline the main differences between parental and hybrids embryos.

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References

Baudoin JP, Silué S, Geerts P, Mergeai G, Toussaint A (2004) Interspecific hybridization with *Phaseolus vulgaris* L.: embryo development and its genetics. Genet Breed 1:349–364

Buishand TJ (1956) The crossing of beans (*Phaseolus* sp.). Euphytica 5:41–50

Debouck DG, Smartt J (1995) Beans, *Phaseolus* spp. (*Leguminosae-Papilionideae*). In Smartt J, Simmonds NW (eds.), Evolution of Crop plants. 2nd ed. pp. 287–294

Geerts P, Toussaint A, Mergeai G, Baudoin JP (2002) Study of the early abortion in reciprocal crosses between *Phaseolus vulgaris* L. and *Phaseolus polyanthus* Greenm. BASE 6:109–119

Lecomte B, Longly B, Crabbe J, Baudoin JP (1998) Etude comparative du développement de l'ovule chez deux espèces de *Phaseolus: P. polyanthus* et *P. vulgaris*. BASE 2:77–84

Ruzin SE (1999) Plant microtechnique and micrmicrocospy. New York: Oxford University Press

Sabja AM, Mok DWS, Mok MC (1990) Seed and embryo growth in pod cultures of *Phaseolus vulgaris* and *P. vulgaris* × *P. acutifolius*. HortScience 25:1288–1291

Yeung EC (1980) Embryogeny of *Phaseolus* : the role of the suspensor. Z Pflanzenphysiol 96:17–28

Yeung EC, Meinke DW (1993) Embryogenesis in angiosperms: development of the suspensor. Plant Cell 5:1371–1381