



Vicia ervilia, or bitter vetch, is a neglected cleistogamous leguminous plant domesticated during the Neolithic period. Initially used for human consumption, over time it was cultivated as fodder crop. Due to the EU's aim to reduce reliance on imported soy, there is interest in using alternative protein sources, as bitter vetch, for feed formulations. Due to lodging problems and creeping habitus of bitter vetch Italian germplasm, a considerable number of seeds is lost during mechanical harvesting. One possible way to circumvent these yield losses is to breed and transfer the erect growth habit trait to high seed yielding genotypes.

Methodology

- ❖ set up a protocol to combine traits of Turkish landraces (erect growth habit) with Italian landraces (high seed yield).
- ❖ overcome the existing cross hybridization barriers among *V. ervilia* genotypes → detailed cytological analysis to determine optimal emasculation and pollination stages
- ❖ SSR markers to verify hybrid status of F1 and F2 progeny

Result 2

Fertility: Of the 5 hybrids, 3 were fertile and produced F2 seeds

Table 1 Attempted crosses between accessions of *V. ervilia* of Turkish and Italian origin

♀	♂	Failed crossing attempts	Pods obtained	F1 Seeds obtained	Successful F1 hybrids	F2 early seeds	F2 late seeds
Turkish	Italian						
8	23	2	1	2	—	—	—
10	21	1	1	1	—	—	—
10	22	1	1	2	—	—	—
10	23	1	4	8	1	10	—
14	21	2	2	5	2 ^a	23	13
14	22	2	3	3	—	—	—
14	23	3	3	4	—	—	—
Italian	Turkish						
21	8	8	1	3	—	—	—
21	14	14	1	3	—	—	—
22	8	9	2	2	—	—	—
22	10	5	3	8	—	—	—
22	14	7	6	10	1	sterile	—
23	8	5	1	1	1	80	32
23	14	8	1	3	—	—	—
total	68	30	55	5	113	45	

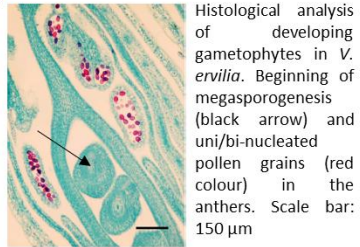
^aone of the two F1 hybrids of the 14×21 cross was sterile, whereas the other hybrid produced 23 seeds



Perspective

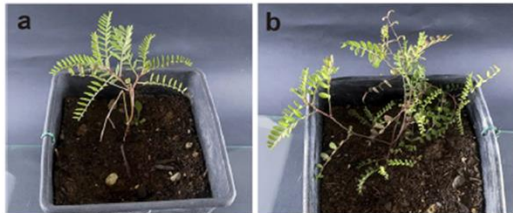
Successful hybridization demonstrated the possibility of exploiting genetic diversity in *Vicia ervilia*, potentially enhancing its agronomic value. Further breeding programs can focus on these hybrids to develop new cultivars with improved traits.

Result 1



Histological analysis of developing gametophytes in *V. ervilia*. Beginning of megasporogenesis (black arrow) and uni/bi-nucleated pollen grains (red colour) in the anthers. Scale bar: 150 µm

Crossing Success: Out of 98 crosses, 55 seeds were obtained, yielding 5 hybrid plants

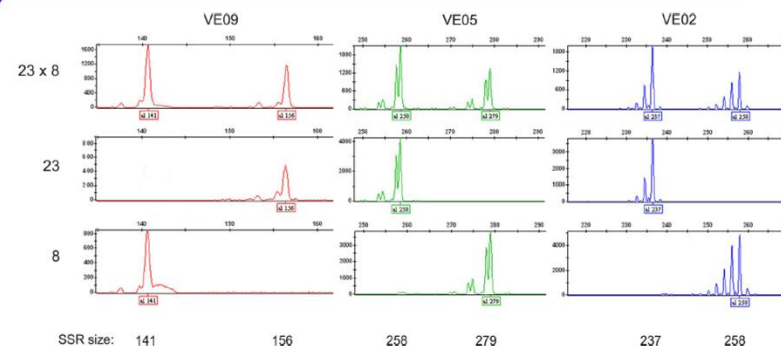


Phenotypic analysis of *V. ervilia* parental lines and their F1 hybrids. Five-month-old plants in pots from seeds grown in greenhouse obtained by self-fertilization of the single stem Turkish accession 14 (a) and the three-stem Italian accession 23 (b). Seven-month-old plants in pots (c) from seeds obtained by self-fertilization of the Turkish accession 14 (maternal parent, at left), by self-fertilization of the Italian accession 21 (paternal parent, at right), and by 14 × 21 cross (F1 hybrid, in the centre).

Development of male and female gametophytes:

Microscopic analysis were undertaken to seek a correlation of macrogametogenesis and stigma receptivity fulfillment with external morphology and dimension of the flowers. We noted that male meiosis in bitter vetch preceded megasporogenesis and we then tried to find the correlation between stigma receptivity and external floral morphology

Result 3



SSR analysis: An example of SSR profile of parental and hybrid pattern is shown. The two parental lines, 23 and 8, were polymorphic and homozygous for all three SSR (VE09, VE05 and VE02) loci, whereas a plant derived from their cross pollination was heterozygous for the same markers.

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Bibliography
The results of this work have been published in: Caceres, M.E., Rubini, A., Russi, L. et al. Obtainment of intraspecific hybrids in strictly cleistogamous *Vicia ervilia* (L.) Willd.. *Euphytica* 220, 111 (2024). <https://doi.org/10.1007/s10681-024-03371-w>