

**P 22: Hybrid-breeding of medicinally used valerian (*Valeriana officinalis* L. s.l.).
A possible concept developing new varieties?**



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Abstract

The aim of this work was to develop and verify a new concept for breeding new hybrid-varieties of valerian without a male sterility system. For this the cross-pollination rate and the performance of inbred plants must be determined.

Keywords: hybrid-variety, inbreed, inbred line, cross-pollination, open pollination

Introduction

The breeding program at the Bavarian State Research Center for Agriculture (LfL), started in 2008, aims to improve production profitability of the German medicinal plants production. Through selection and cross-breeding, coarser rooted valerian cultivars should be developed, so that high human and technical effort during the valerian harvest and postharvest process can be reduced. At the same time the quality requirements of the European Pharmacopoeia shall be safely kept.

The new hybrid-concept by valerian

The currently in cultivation used valerian varieties are populations from pure selective breeding (mass selection) or cross-breeding. In this context, detailed information about the extent of self-, respectively cross-pollination is not found in literature. The population varieties show usually unwanted high variability in many characteristics (e.g. ingredients contents in different years or morphological differences between plants of the same variety). Hybrid breeding has in addition to the aspect of heterosis, precisely the advantage of homogenization. Only varieties with reliably high homogeneity can achieve the above mentioned requirements.

For a reliable hybrid-system one condition have to be fulfilled, a hybrid-mechanism must exist. Manual crosses are not practical to produce a sufficient amount of hybrid-seeds, the use of gametocides seems not to be acceptable for medicinal plants and applicable male-sterility systems are not known for valerian. Therefore, a new approach has been developed. The parental components should produce seeds by open pollination (random mating) and thereby the hybrid-seed will be produced by using the occurring cross-pollination of valerian. Certainly, the harvested seed lot will contain a certain fraction of inbreed seeds. This fraction must be kept at a tolerable amount. It is assumed, that seeds respectively plants arisen by selfing usually show lower vitality and a reduced growth rate (BERNÁTH, 1997), and will be either suppressed or compensated by the hybrid plants in the established field crop.

The outstanding issues

The presented concept results in the following questions. A) What is the percentage of cross-pollinated seeds respectively inbreed seeds in cross-pollinated populations? B) Is for inbred lines an inbreeding depression identifiable or, in other words, exhibit inbred plants lower vitality?

Materials and Methods

A) Determine the cross-pollination rate

Non-systematic studies have been conducted, in which two valerian origins that are adjacent in the LfL-stock and blooming at the same time were incurred by Amplified-Fragment-Length-Polymorphism (AFLP). The AFLP analysis was conducted with young leaf material of all examined

existing plants and individuals from F1-population originated by open pollination (random mating) with more than 80 valerian populations of the LfL-stock. Molecular primers were applied, which have shown a variety of polymorphisms in a previous study with valerian (HEUBERGER et al. 2012). The AFLP analysis was carried out according to an established protocol for hops including a few modifications.

After these first findings, a more systematic study was started, of which the first results are presented here. 16 valerian plants (elites) were transferred in *in-vitro* for cloning using side shoots. At the same time the inbred lines were generated from the elite plants. The first generations (I1) were examined for homozygous present DNA-based marker bands (AFLP). Six elites could be found that differ in at least one homozygous band. The clones of the selected elites were used in different pair combinations based on their specific polymorphism characteristics.

The mating clones were planted together at two locations for two years. The planting corresponds to a poly-cross-system, so that an equal pollination (with foreign pollen) is possible. The seeds were taken separately from the plants in the center plot and purified according to the seed-treatment protocol of the LfL. One seed drilling was done by hand in multipot-culture-plates in the greenhouse. At the 3-4 leaf-stage of the plants, young leaf material of nearly 340 plants was sampled and analyzed using the AFLP-method as described by Heuberger et al (2012).

B) Estimate of inbreeding depression

As part of the breeding work, many inbred lines were developed. Thereby, single plants were selected from the inbred lines; these bloomed isolatedly, so that inbreed-seeds were produced. In several steps, such inbred lines with different grades of inbreeding (I1, I2, I3 and I4) could be generated. At each level of inbreeding a performance test was carried out on the field, were the characteristics e.g. vitality during germination and seedling cultivation, crop coverage and vitality in field, as well as rootstock weight was recorded.

Results

A) Determine the cross-pollination rate

Figure 1 shows the AFLP-result of the initial investigation on two valerian origins of the LfL-stock. The descendants were generated by cross-pollination for 100 % (F1-population A) and for 67 % (F1-population B).

From the poly-cross combinations of the second approach, the AFLP banding patterns of the F1 descendants from different combinations and two locations indicated different cross-pollination rates. Figure 2 shows the two evaluable combinations (combination A and B1/B2) with averaged cross-pollination rates of 93.4 % and 79.5 %. Remarkable are the rates of combination A at the location A. However, no significant differences were found (Welsh-t-Test p-value = 0,644).

B) Estimate of inbreeding depression

Inbred lines with different genetics and degree of inbreeding were analyzed for three years. The generation I3 shows poorer performances in the characteristics vitality during germination and seedling cultivation, crop coverage and vitality in field, as well as rootstock than inbred lines at the first generation (I1). Figure 3 shows this relationship for the example vitality during germination and seedling cultivation.

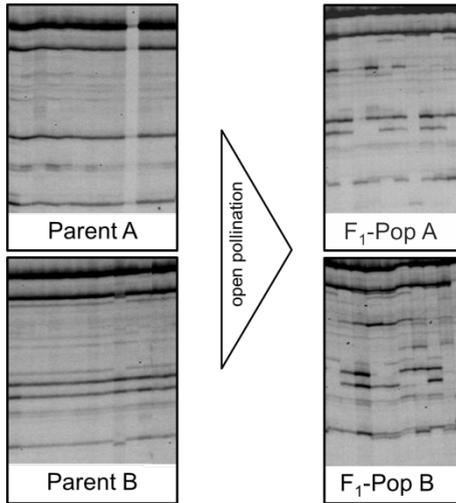


Fig. 1 Cross-pollination in open pollinated populations (Top-cross). Left: the AFLP band patterns of the seed bearer (Parents); Right: ALFP band patterns of the respective descendants (F1-populations).

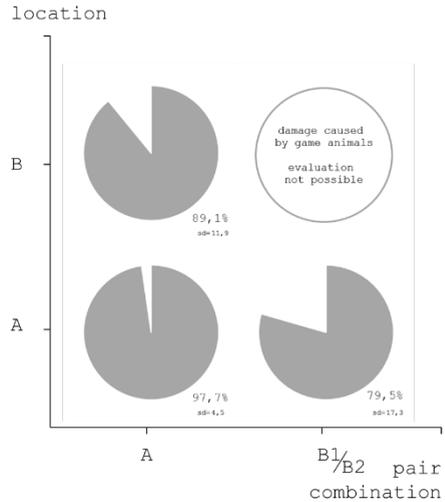


Fig. 2 Second approach of cross-pollination in open pollinated populations (Top-cross). The averaged cross-pollination rates from two locations (Y-axis) and two pair combinations (X-axis) are shown. (sd = standard deviation).

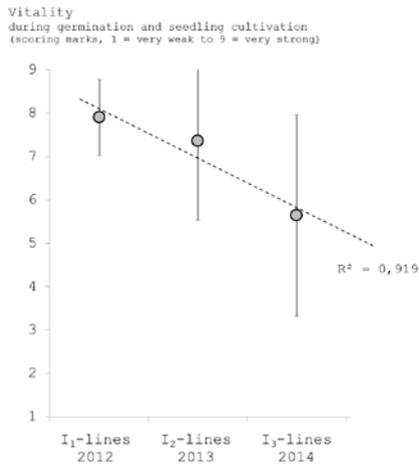


Fig. 3 Development of the vitality during germination and seedling cultivation of three inbred line generations. Vertical lines present the standard deviation, broken line present the trend line with the coefficient of determination (R^2).

Discussion and conclusion

As reflected in the introduction, the conditions for establishing hybrid varieties are a hybrid-mechanism and the lower performance of inbred descendants. The high cross-pollination rates observed in the first experimental year could serve for this. Since wide variation in the cross-pollination rate occurred occasionally within the tested combinations, the second experimental year should be evaluated before final conclusions. It should be noted, that basically the validity applies only for the tested combinations. Most likely, other lines will behave similarly; however, in the end, the cross pollination rate of the developed hybrid variety must be verified. For this a working method using AFLP has now been established, after other approaches using isoenzyme polymorphisms have failed (PENZKOFER et al., 2014).

An occurring inbreeding depression is not surprising, but it was confirmed in valerian again. Thus, the second condition for the new concept would be also met, that is that a hybrid variety from open pollination, without male-sterility system will serve the needs of field cultivation. The study also supports previous information that the underlying pollination type of valerian is obligate-facultative cross-pollinated (HEEGER, 1956; BERNÁTH, 1997). This means, valerian prefers cross-pollination, however autogamy is possible. The latter is important for the development of hybrid varieties, because hybrid-breeding can only be exploited fully, if inbred lines are used (BECKER, 2011).

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