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Short Communication

Inheritance of calyx shape in the genus *Origanum* (Lamiaceae)

J. Novak, D. Gimplinger and Ch. Franz

Institute for Applied Botany, University of Veterinary Medicine, Veterinärplatz 1, A–1210 Vienna, Austria;
E-mail: Johannes.Novak@vu-wien.ac.at

With 1 table

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Abstract

Five different traits responsible for calyx shape within the genus *Origanum* were found to be determined by five independent genes. The five traits are bell-shaped vs. tubular-shaped calyx, angular vs. round teeth on the upper lip, entire vs. denticulate upper lip, fully developed vs. reduced lower lip and entire vs. denticulate lower lip. The simple Mendelian inheritance of most traits for calyx shape could be used to identify hybrids between *Origanum* species arising from open-pollinated plants and so avoid tedious artificial pollination resulting from small flower sizes in *Origanum*. Such hybrids are interesting base material for character transfer (e.g. winter hardiness or seed size) between economically important species.

Key words: *Origanum majorana* — *Origanum vulgare* — calyx shape — interspecific hybridization

The genus *Origanum*, which is divided into 10 sections, contains two important aromatic plants, marjoram and oregano, with different sensorial qualities. The aromatic quality of marjoram is to be found only in one species in the section *Majorana* (*Origanum majorana*). In contrast to marjoram, the quality of oregano depends upon many different species (even of other genera of the Lamiaceae and other plant families). The best quality oregano ('Greek oregano'), however, arises mainly from the section *Origanum* within the genus *Origanum*.

Both marjoram and oregano contain an essential oil with monoterpenes and sesquiterpenes responsible for their use as aromatic plants. The bicyclic monoterpene *cis*-sabinene hydrate and its acetate derived from the 'sabinyl' biosynthetic pathway are responsible for marjoram's quality, while the phenolic monoterpene carvacrol, arising from the 'cymyl pathway' (Skoula et al. 1999), is the typical compound of oregano. The genus is regarded as taxonomically complicated, but since the taxonomic revision of Ietswaart (1980), listing 39 species and 16 naturally occurring hybrids, our knowledge of the taxonomy of the genus *Origanum* has increased significantly. In Ietswaart's (1980) revision, the shape of the calyx has a dominant role in distinguishing species. The calyx shape is very variable between, but stable within species.

In the sections *Elongatispica, Origanum* and *Prolaticorolla,* the calyces are regularly five-toothed for approximately one-third of their total length and tubular. In the section *Majorana,* the calyces are one-lipped, flattened and of an angulate, obovate form. Within the numerous naturally occurring hybrids between *Origanum* species, huge variability with intermediate shapes of calyces has been found (Ietswaart 1980). This hybrid variability in calyx shape was described by Appl (1928), who found three genes to be responsible for calyx shape traits in offspring following naturally occurring hybridization between *Origanum majorana* and *Origanum vulgare*. Kokkini and Vokou (1993) also found the shape of calyces of a natural hybrid (*Origanum* × *intercedens*) to be intermediate between the presumed parents *Origanum onites* (section *Majorana*) and *Origanum vulgare* ssp. *hirtum* (Link) Ietswaart. The shape of the calyx could therefore be a morphological marker to identify interspecific crossings.

Plant materials: a naturally occurring F_1 hybrid between *O. vulgare* ssp. and *O. majorana*, identified by the intermediate shape of the calyx, was selfed in the greenhouse during the winter of 1997–98. A total of 82 plants from the resulting F_2 generation were maintained in pots and kept in the greenhouse until seed ripening.

Determination of the calyx shape: during flowering, flower heads were sampled from each plant. Three calyces per plant were separated from the flower heads and digitized with a WILD M3Z microscope (Heerbrugg, Switzerland) and a JVC TK1070E camera (LB-electronics, Vienna, Austria) at a magnification of ×3.25. The length and width of the calyx parts were measured with the image analysis software 'NIH-Image' (Wayne Rasband, NIH, housepage: vsb.info.nik.gov/nik-image/ software in public domain) on an Apple Performa 630. The shape traits were described according to Ietswaart (1980) and classified either as 'marjoram type' (for a calyx shape as in the section *Majorana*) or 'oregano type' (for a calyx shape corresponding to the sections *Elongatispica*, *Origanum* and *Prolaticorolla*).

The shape of the calyx in *Origanum* involves at least five different traits. Each of these five traits is controlled by a single gene (Table 1), thus confirming the results of Appl (1928) but two more genes were described. However, in some traits (especially in the trait 'lower lip fully developed' vs. '5-toothed' (Appl 1928)), a direct comparison was not easy to make because of the different descriptions formerly used.

In three out of the five traits, i.e. bell vs. tubular calyx shape and the degree of connation of the individual calyx leaves to form either an entire or denticulate upper or lower lip, the marjoram form was dominant. For the other two traits, i.e. shape of calyx teeth and reduction of lower lip, the typical oregano form was dominant. Therefore, the traits were grouped according to this distinction to test for possible

Table 1: Segregation of five calyx shape traits

'Marjoram type' : 'oregano type'	Abbreviation	Suggested segregation	Observed	χ^2	P
Bell-shaped calyx : tubular-shaped calyx	B : b	3 : 1	58 : 24	0.796	0.372
Upper lip entire : upper lip denticulate	U : u	3 : 1	64 : 18	0.407	0.524
Lower lip entire : lower lip denticulate	E : e	3 : 1	60 : 22	0.146	0.702
Round calyx teeth : angular calyx teeth	t : T	1 : 3	25 : 57	1.317	0.251
Lower lip reduced : lower lip fully developed	l : L	1 : 3	20 : 62	0.016	0.899
BUE : BuE : bUE : BUe : Bue : bUe : buE : bue		27 : 9 : 9 : 9 : 3 : 3 : 3 : 1	35 : 14 : 6 : 13 : 3 : 2 : 6 : 3	6.118	0.526
TL : Tl : tL : tl		9 : 3 : 3 : 1	46 : 11 : 16 : 9	4.201	0.241

linkages using two models, one with the three 'marjoram'-dominant genes and the other with the two 'oregano'-dominant genes (Table 1). The results proved the existence and independence of five single genes for the respective five traits.

More than the five genes described here will influence the calyx shape, resulting in large variability in the F_2 generations of interspecific hybrids. However, apart from these five traits, it was difficult to classify other traits or to determine simple Mendelian segregation.

Although oregano and marjoram, based on their monoterpene composition, represent two completely different qualities in the market, gene transfer by hybridization is an interesting plant breeding tool for these species. Marjoram, for example, is a perennial plant, but not winter hardy in colder climates. Some species of oregano, however, are very hardy. The transfer of winter hardiness to marjoram was the aim of Appl (1928) and of Dzevaltovskii and Polishchuk (1975). While Appl (1928) used the calyx shape as a morphological marker, the main morphological marker used by Dzevaltovskii and Polishchuk (1975) was the colour of the flower. In this latter example, it was the hybridization of white-flowering *O. majorana* with purple-flowering *O. vulgare* ssp. *vulgare*, a widespread species which, in contrast to other Mediterranean subspecies of *O. vulgare*, contains only minor quantities of essential oils (Kokkini et al. 1994). The colour of the flower is fixed in *O. majorana* and *O. vulgare* ssp. *vulgare*, but varies in other subspecies of *O. vulgare* and so this marker is suitable only in restricted cases. Furthermore, a preferred gene donor should already have a high essential oil content to avoid segregation for this characteristic during the following generations. Such donors would still be preferred, even if they also contained large amounts of undesirable compounds from the 'cymyl pathway', such as carvacrol, thymol, *p*-cymene or γ-terpinene. It is easier to select against this pathway by using the human nose as a selection tool, than to select for a higher content of essential oil which would require time- and labour-consuming chemical analyses. This approach of using the human nose as a selection tool was applied for selecting the now sensorially homogeneous and 'pure sabinyl' marjoram from heterogeneous natural populations (Novak et al. 2002). Moreover, the gene donors' higher variability in essential oil content may even be used to further increase marjoram's essential oil content.

Furthermore, the higher 1000-grain weight of marjoram, which amounts to 0.2 g (Dachler and Pelzmann 1999), might be transferred into oregano, which generally has a very low seed weight. Thanos et al. (1995) determined a 1000-grain weight of only 0.061 g in, for example, *O. vulgare* ssp. *hirtum*. A greater seed weight could enhance the still problematic direct sowing of oregano which, due to an increased demand for its essential oil, is at the edge for large-scale cultivation.

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