

Self-incompatibility and male sterility in six *Matricaria recutita* varieties

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Summary

On purpose to develop suitable mother lines for a large scale hybrid production of the medicinal crop species *Matricaria recutita* [L.] Rauschert investigations to

- determine the proportions of self and cross fertilization,
- identify self-incompatible genotypes and
- observe spontaneous or induced male sterility

were undertaken. The seed production per flower head under isolated and under untreated control-conditions was evaluated in six German chamomile varieties. Under assumption of equal behaviour of plants concerning their reproduction rate results showed opposed reactions of the auto-tetraploid ‘Manzana’ and the diploid ‘Degumille’ to isolation. Moreover, slightly significant interactions between cultivars and treatment concerning seed production per flower head indicate the influence of isolation being cultivar specific. An average of 22 % of self-fertilization under open pollination conditions is displayed over all cultivars. Highly significant differences between cultivars reveal cultivar specific self-incompatibility, at which the diploid varieties ‘Bona’ and ‘Degumille’ exhibit the highest levels of 25 % and 28 %, respectively. 9 of 1105 plants showed spontaneous single flower head aberrations indicating possible tendencies to male sterility. Spontaneous pollen sterility, estimated after aceto-carmin staining, reached mean levels between 0.5 % (‘Bona’) and 2.4 % (‘Lutea’). The progeny after crossings across all six varieties showed an average of 10.20 % pollen sterility indicating a tendency of increasing pollen sterility by intra-specific crossings. Nevertheless, as the best way to raise suitable mother lines the development of vegetatively propagated self-incompatible diploid genotypes can be recommended.

Introduction

German chamomile (*Matricaria recutita*) is known to be a mainly out-crossing plant (MASSOUD, 1988; CORTNUMMÉ, 1980; LETCHAMO, 1992), but exact values of proportions of cross- and self-fertilization are not available and also might differ by variety and/or ploidy level (GRUBE, 2009). The incidence of self-incompatibility and the maintenance and propagation of self-incompatible genotypes is an appreciated basis for successful artificial crossings for hybrid breeding. Prior investigations on seed development after cross- and self-fertilization in allopolyploid *Achillea*-species (*Asteraceae*) showed a high rate of self-incompatibility (VETTER et al., 1998; JEZEK, 1989). For *Matricaria* no reliable data are available. Under the aspect of raising suitable mother lines for hybrid breeding naturally occurring and induced male sterility is sought (FAEHRICH et al., 2012). As the chamomile’s flower head is build up by hermaphroditic disc flowers and female ray flowers (MASSOUD, 1988; CORTNUMMÉ, 1980), naturally occurring aberrations with a higher ray flower/ disc flower ratio indicate tendencies towards male sterility. The same does increased pollen sterility. Artificial inter-cultivar crossings could, by genetic alteration, change this reproductive trait (BECKER, 1993).

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Material and methods

Six *Matricaria recutita* [L.] Rauschert varieties were included in these recent trials, comprising three diploid ones (‘Bona’, ‘Degumille’ and ‘Hungarian 2’) and three colchicine induced auto-tetraploid ones (‘Manzana’, ‘Lutea’ and ‘Hungarian 1’). While the accessions ‘Hungarian 1’ and ‘Hungarian 2’ were supplied by the University of Budapest, Faculty of Horticultural Sciences, all of the other varieties have been maintained in own experimental stations of the University of Veterinary Medicines, Vienna, Austria for about 20 years. ‘Manzana’ is a protected variety since 1986, ‘Degumille’, ‘Bona’ and ‘Lutea’ are described, discrete varieties since 1977, 1984 and 1995, respectively (HEINE et al., 2009). Purity of defined ploidy level in distinct varieties was assessed by flow cytometry of 1800 individuals (300 per variety) and ranged from 81 % (‘Hungarian 1’) to 100 % (‘Bona’). Only ‘Lutea’ exhibited lower ploidy purity with 61 % of 4x-plants (detailed data not shown).

To prove self-incompatibility and the rate of self-/cross pollination 300 plants were included in a trial with all six above mentioned varieties and two treatments. Ploidy levels of these plants were assumed to be according to their origin and the performed ploidy level testings. All of the tested plants were raised from the same batch of seeds as the above mentioned plants. Treatments included isolation with micro-perforated crispac bags covering whole single plants and untreated, open pollination control conditions. Both versions took place in the same green house, under the same environmental growing situation. 25 plants were kept per factor combination (variety and treatment). The number of seeds per flower head was assessed by counting visually as fertile determinable fruits (achenes, slightly curved, with a length of 1-2 mm and a width of app. 0.3 mm, striped) under light microscope. In case of the control version one flower head per plant, in case of isolation up to three flower heads per plant after frequent hand self-pollination were assessed. If all three flower heads did not produce any seeds, the plant was defined as a self-incompatible plant. To determine self pollination against cross pollination always the first examined flower head was taken into account.

To assess potentially male sterile plants on the one hand 1105 plants deriving from the above mentioned varieties (100 - 300 plants per variety) were observed regarding the occurrence of flower head aberrations like missing hermaphroditic disc flowers or additional rings of female ray flowers. Both effects increase the female/hermaphroditic floret rate per flower head. From all suspicious flower heads seeds were sown to raise and examine the F₁-generation. On the other hand pollen fertility was estimated under light microscope after aceto-carmin staining of patted-off mature pollen of one flower head per plant, using again a trial set of 25 plants per accession under open pollination conditions in the green house. Additionally 150 diallele reciprocal artificial crossings between all varieties were undertaken to compare the rate of pollen sterility/fertility after inter-cultivar crossing.

Statistical analysis included analysis of variance with one factor (cultivar) for both treatments (isolated and control) in terms of produced seeds per flower head as well as analysis of variance with two factors (cultivar and treatment) over all data to reveal different behaviour of chamomile cultivars under isolation. To compare

ratios of self-/cross pollination among cultivars, means of produced seeds per flower head under isolation were calculated as percentages of mean amounts under control conditions and tested against average equal values for all cultivars/varieties per chi-square-test after weighting the percentages. A similar procedure was performed to gain results concerning self-incompatibility (comparing percentages of self-incompatible plants per accession) and potential ms-(male sterile) plants (comparing percentages of relevant flower head aberrations per cultivar). To assess occurring pollen sterility 150 data of plants grown under normal green house conditions of six varieties (25 replications per variety) were compared with data from the progeny (35 plants) after intra-specific crossings using univariate analysis of variance and t-test.

Results

Comparing the means of seeds per flower head between cultivars/varieties within different treatments results show no significant influence of cultivars (p -value for control = 0.108, for isolation = 0.119), but post-hoc-tests (Duncan) show 'Manzana' (4x) and 'Degumille' (2x) belonging to different homogenous groups. Under open pollination (control) conditions 'Manzana' exhibits the lowest amount of produced seeds (mean of 20.40 seeds), while 'Degumille' shows the highest amount (mean of 58.27 seeds). Under isolated conditions the proportion is exactly reversed, i.e. 'Degumille' shows the lowest (mean of 3.46 seeds), 'Manzana' the highest amount of seeds per flower head (mean of 13.94 seeds). The diploid, and therefore rather original or natural cultivars 'Bona' and 'Degumille' (the tetraploid cultivars 'Manzana' and 'Lutea' are colchicine-induced polyploids) exhibit two very differing columns of means of seeds per flower head (43.05 vs. 8.50 for control and isolation for 'Bona' and 58.27 vs. 3.46 for 'Degumille', resp.), i.e. under isolation the seed production drops very severely in diploids. 'Manzana' (4x) does not produce so many seeds under normal (control) conditions but is rather insensible to conditions of isolation (20.40 vs. 13.94) (Fig. 1).

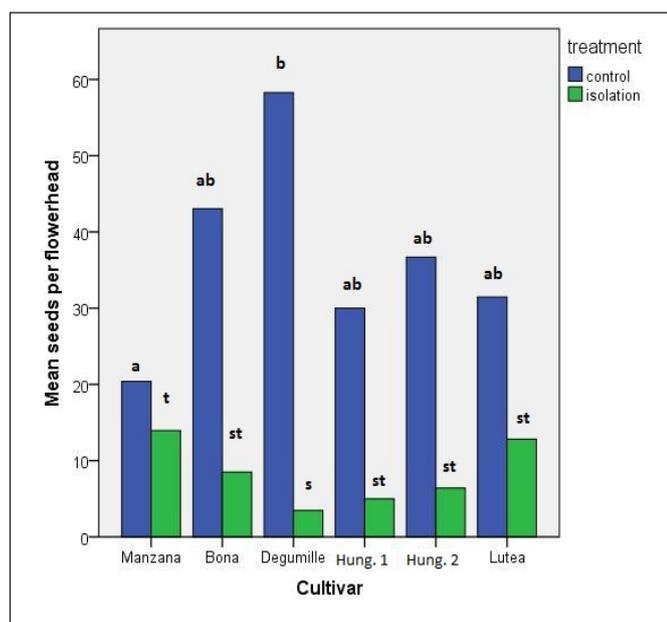


Fig. 1: Comparison of seed production between cultivars/varieties and treatments, different letters indicate statistical difference at the $p < 0.05$ level

Conducting analysis of variance with two factors (cultivar and treatment) over all data a significant influence of treatment ($p = 0.000$) and a slight significance of interactions between cultivar and treatment ($p = 0.025$) comes out. Both calculations indicate the influence of isolation being cultivar specific. At this stage we cannot reveal whether this influence derives from different sensitivities to stress situations like isolation bags or from genetically caused self sterility effects.

Open pollinated control plants perform self- and cross-pollination; isolated plants can only perform self-pollination. Just based on these considerations, percentages of self- and cross-pollination relying on the means of number of seeds per flower head were calculated. The same reproductive behaviour of plants of the same variety and ploidy level under isolation and under 'normal' conditions was assumed. Results show an average of 22% self-pollination under open pollination conditions with a significant influence of cultivar ($p = 0.000$, Fig. 2).

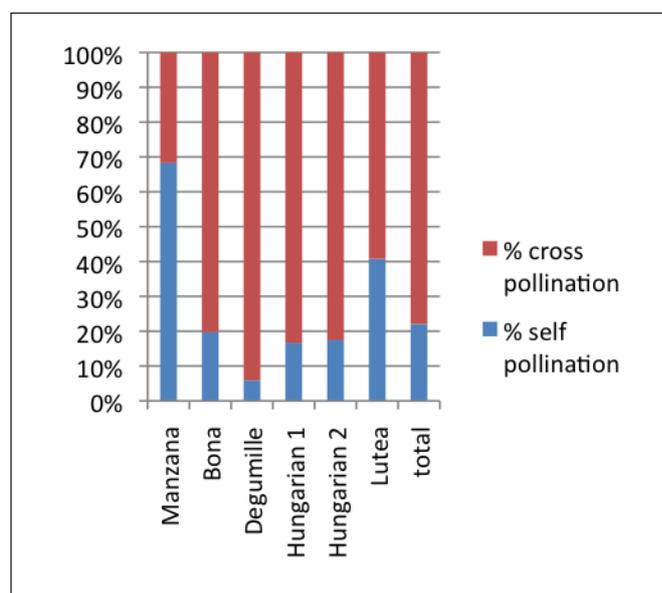


Fig. 2: Calculated percentages of self- and cross-fertilization under open pollination conditions, based on the means of produced seeds per flower head under isolation and under open pollination conditions

Self-incompatibility was defined as 3 examined flower heads in succession per isolated plant without any seed production despite of frequent hand pollination. Tab. 1 shows the number and percentages of measured and as self-incompatible determined plants in six varieties. Tested by chi-square-tests a statistically highly significant difference between cultivars can be detected ($p = 0.000$).

Remarkable is the high self-incompatibility of the diploid cultivars 'Bona' and 'Degumille' with almost 28 % and 25 %, respectively.

To reveal tendencies to develop male sterility (ms), relevant flower head aberrations (9 of 1105 plants) were labelled and counted. No significant difference between cultivars ($p = 0.564$) did appear, although 'Bona' and 'Degumille' (both diploid) seem not to tend to flower head alterations (Tab. 2). The F_1 -generation of the selected flower heads was raised, but no renewal of relevant flower head alteration did occur.

Pollen sterility data of the parental generation showed no significant influence of cultivar ($p = 0.518$) and no completely pollen sterile plant appeared in the parental generation. Mean percentages of pollen sterility ranged from 0.55 % ('Bona') to 2.20 % ('Lutea') (Fig. 3). Compared with the F_1 -generation after crossings across dif-

Tab. 1: Detected percentages of self-incompatibility in six chamomile varieties

Cultivar	3x0 seeds-plants	of measured	% of self incompatible plants
Manzana	1	16	6.3
Bona	5	18	27.8
Degumille	6	24	25.0
Hungarian 1	0	17	0.0
Hungarian 2	1	15	6.7
Lutea	4	23	17.4
total	17	113	15.0

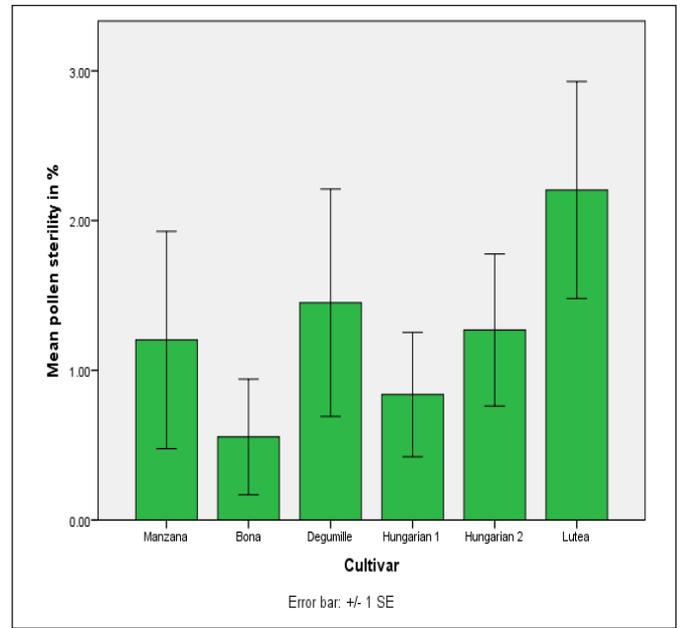
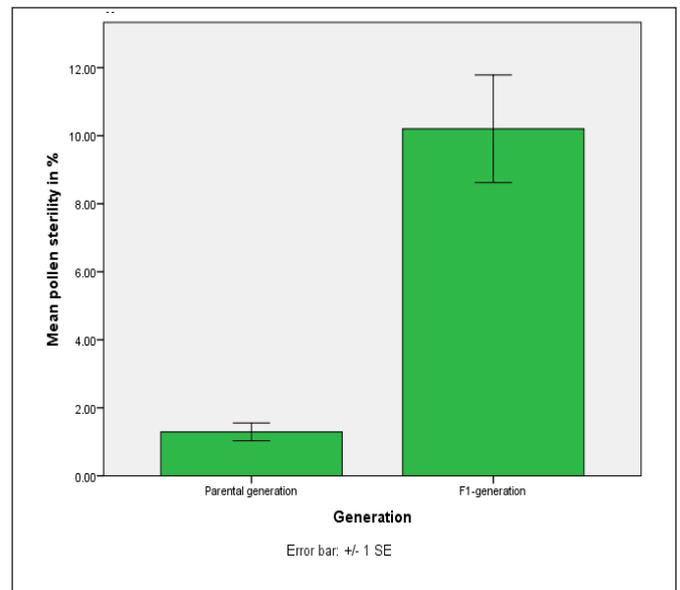
Tab. 2: Detected percentages of plants with flower head aberrations indicating tendencies to male sterility in the parental generation of six chamomile varieties

Cultivar	Plants with potential ms-formations	of measured	% of potential ms-plants
Manzana	3	300	1.0
Bona	0	100	0.0
Degumille	0	100	0.0
Hungarian 1	0	100	0.0
Hungarian 2	1	205	0.5
Lutea	5	300	1.7
total	9	1105	0.8

ferent varieties significant differences in pollen sterility ($p = 0.000$) could be detected, although also in the F_1 -generation no completely sterile plant occurred. The parental vs. the F_1 -generation displayed mean pollen sterility values of 1.29 % and 10.20 %, respectively (Fig. 4 and 5). Details of mean pollen sterility of progeny in comparison with their respective parental cultivars are shown in Tab. 3. The data indicate the increase of pollen sterility by inter-cultivar crossings.

Discussion

Looking at figure 1 not only the cultivar-specific reaction to isolation can be discovered but also – knowing that ‘Degumille’ and ‘Bona’ are diploid and ‘Manzana’ and ‘Lutea’ auto-tetraploid – a ploidy level dependant behaviour concerning reproductive traits under isolation can be assumed. Obviously diploids suffer more from isolation while tetraploids in general produce fewer seeds but their reaction to forced self-fertilization is not dramatic. Whether this phenomenon derives from a general lower ability of diploids to cope with stress situations or from a genetic disposition of diploids to – as far as possible – avoid selfing cannot be proven in these investigations, because no other trials on stress-situations were performed. However, in various publications decreasing seed production and lower self-incompatibility is stated for polyploid individuals (GRUBE, 2009; BECKER, 1993). The increased adaptability of polyploids to extreme environmental conditions is explained as a result of higher genetic variability and therefore a higher probability to produce suitable genotypes (KUCKUCK et al., 1985; SINGH, 1992). Genomic rearrangements and instability in plant polyploids play a role to develop

**Fig. 3:** Mean percentages of pollen sterility in six varieties of the parental generation**Fig. 4:** Mean percentages of pollen sterility in parental vs. F_1 -generation after crossings across different varieties

this high variability (CHEN et al., 2006; WANG et al., 2010). As one of the most important causes for decreased fertility in polyploids altered physiologic features due to the increase of cell size is postulated (KUCKUCK et al., 1985).

Provided that the reproductive behaviour of chamomile plants under open pollination condition resembles to isolated, but apart from that identical conditions, proportions of self- and of cross pollination in different varieties can be assumed (Fig. 2). On the other hand one could also assume a mainly out-crossing plant being forced to do selfing will display a higher fertilization rate and seed production by self pollination than under open pollination conditions where cross-fertilization is possible. No other stress-tests to discover stress

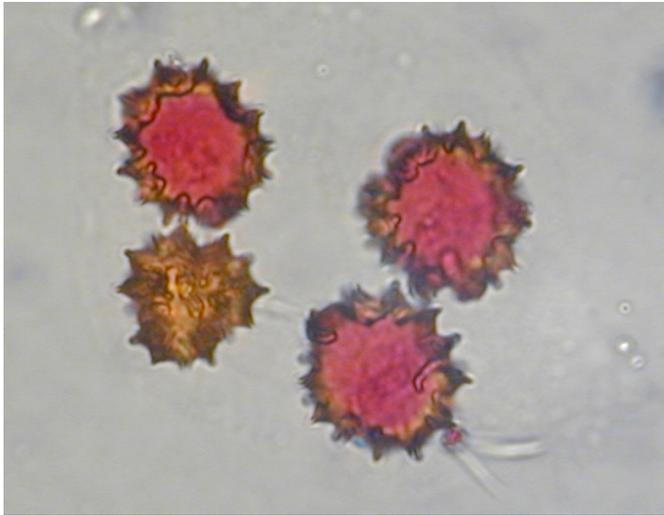


Fig. 5: Infertile pollen (yellow, in the lower left corner) vs. fertile pollen (red, the other three) in the F₁-generation

Tab. 3: Detailed percentages of pollen sterility of parental cultivars and their progeny, respectively

Cultivar of mother	pollen sterility in %, mean of cultivar	Cultivar of father	pollen sterility in %, mean of cultivar	pollen sterility in %, mean of progeny
Manzana	1.20	Lutea	2.20	9.93
Manzana	1.20	Hung. 1	0.84	n/a
Manzana	1.20	Hung. 2	1.27	n/a
Lutea	2.20	Hung. 1	0.84	8.82
Lutea	2.20	Manzana	1.20	4.76
Hung. 2	1.27	Bona	0.55	8.20
Hung. 2	1.27	Degumille	1.45	7.58
Hung. 1	0.84	Lutea	2.20	8.41
Degumille	1.45	Bona	0.55	4.21
Bona	0.55	Degumille	1.45	15.53
Bona	0.55	Hung. 2	1.27	20.33

susceptibility of different varieties were performed. Therefore, to be sure about the proportions of self- and cross pollination the performance of trials with phytochemical or molecular markers to assess the parents and their progeny without creating isolation stress would be necessary. Similar studies over five generations with phytochemical markers in chamomile, but with different investigation targets were conducted by MADER (1998). Easily recognizable phenotypic markers are not available in chamomile.

In these present trials the whole referring plant (as one genotype) was put under an isolation bag to assess self compatibility or not, in other trials only single flower heads were isolated (VETTER et al., 1998; CORTNUMMÉ, 1980). Nevertheless, the proportions of self- and cross fertilization show a significant influence of cultivar and a tendency of diploids (e.g. 'Bona' and 'Degumille') to perform low rates of self fertilization. Ploidy level purities within the single cultivars were very high. 'Degumille' (2x) exhibited 98 %, 'Bona' (2x) 100 % according individuals. In continuation of these thoughts it is not surprising that self-incompatibility also presents a significant cultivar-specification, and shows to be high in diploids (Tab. 1). This

message could be of high value for breeders as the chance to develop suitable mother lines for artificial crossings from selected, self incompatible and vegetatively propagated diploid plants seems to be high.

In order to test different options to create suitable mother lines for cross breeding purposes, naturally occurring flower head aberrations indicating the development of male sterility (ms) by performing more ray flowers and/or fewer disc flowers were assessed. After finding 9 of 1105 potential ms-plants (Tab. 2) no renewal of such aberrations occurred, neither in the F₁-generation raised from the seeds of the selected and suspicious flower heads nor among the seedlings of the base plants. This observation suggests these appearances to be only somatic and not genetically determined. An F₂-generation could not be established. The fact that these aberrations only occurred at single flower heads and never concerned the whole plant underlines a similar approach. Therefore the establishment of mother lines via spontaneously occurring ms-plants cannot be recommended without conducting further research. Possibly there could be a chance to generate ms-aberrations by induced mutations (MATSUMARA et al., 2010).

Based on investigations and publications concerning changes of pollen sterility/fertility after inter- and intra-specific crossings and/or in different ploidy levels (LAMBROU et al., 2001, pers. comm.; LÖSSL, 1999; KÖHLER et al., 1991; KEMPE and GILS, 2011; KAUL, 1988) diallele reciprocal crossings between all varieties were conducted and pollen fertility was tested before and after the crossings. Controlled crossings could enhance the chance to develop decreased fertility in general and male sterility in particular (LÖSSL, 1999). This effect could have its origin in a disposition of CMS (cytoplasmic male sterility) or could be determined solely genetically (GMS, genetic male sterility). Both cases would have to be proven in numerous progeny tests (KUCKUCK et al., 1985; ODENBACH, 1997). As our trials discovered significant statistical differences in pollen sterility between the two tested generations (parental and F₁-generation), pollen fertility/sterility of cuttings of the F₁-generation will be continued to be tested and female fertility/sterility of the F₁-generation will be assessed by observing seed production and germination rates. Certainly, more investigations have to be undertaken to ensure and specify the effect of intra-specific crossings on male or female fertility.

According to the outcomes of self-incompatibility tests and different focused kinds of male sterility the most appropriate approach to create suitable mother lines for hybrid production seems to be the development of diploid (esp. 'Bona' and 'Degumille') lines from vegetatively propagated self-incompatible plants. Also an approach via pollinating maintainers to establish self-incompatible mother lines – similar to CMS-maintainer lines, according to PANK et al. (2001) – is worth consideration. Further investigations to detect self-incompatibility in an early stage of plant development, e.g. by watching the growth of the pollen tube by light microscope, are recommended.

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References

- BECKER, H., 1993: Pflanzenzüchtung. Ulmer, Stuttgart.
 CHEN, ZJ., NI, Z., 2006: Mechanisms of genomic rearrangements and gene

- expression changes in plant polyploids. *Bioassays* 28, 240-252.
- CORTNUMMÉ, J., 1980: Beiträge zur Züchtung der Kamille (*Matricaria chamomilla* L.). Diplomarbeit, Technische Universität München-Weihenstephan.
- FAEHRICH, B., FRANZ, C., 2012: Effects of gibberellic acid as a gametocide on different genotypes of German Chamomile (*Matricaria recutita* [L.] Rauschert). *J. Appl. Bot. Food Qual.* 85, 73-74.
- GRUBE, M., 2009: Populationen – Biologie, Genetik, Ökologie. Vorlesungsunterlagen SS 2009, Uni Graz.
- HEINE, H., EGER, H., FRANZ, C., BLÜTHNER, W.-D., HOPPE, B., 2009: Kap. 2.3 Sortenwesen. In: SALUPLANTA (Hrsg.), *Handbuch des Arznei- und Gewürzpflanzenbaus*, Band 1, Grundlagen des Arznei- und Gewürzpflanzenbaus I, 609-640. Verein für Arznei- und Gewürzpflanzen SALUPLANTA e.V., Bernburg.
- JEZEK, B., 1989: Kreuzungsversuche an europäischen Vertretern des *Achillea millefolium*-Komplexes. Diplomarbeit, Universität für Bodenkultur und Veterinärmedizinische Universität, Wien.
- KAUL, M.L., 1988: Male sterility in higher plants. Springer, Berlin.
- KEMPE, K., GILS, M., 2011: Pollination control technologies for hybrid breeding. *Mol. Breed.* 27, 417-437.
- KÖHLER, R., HORN, R., LÖSSL, A., ZETSCHKE, K., 1991: Cytoplasmic male sterility in sunflower is correlated with the co-transcription of a new open reading frame with the *atpA* gene. *Mol. Gen. Genet.* 227, 369-376.
- KUCKUCK, H., KOBABE, G., WENZEL, G., 1985: Grundzüge der Pflanzenzüchtung, 5., neubearbeitete und erweiterte Auflage. Walter de Gruyter, Berlin, New York.
- LAMBROU, M., BEIN-LOBMAIER, B., FRANZ, Ch., 2001: Cytological analysis of di- and tetraploid plants of *Matricaria recutita* (Asteraceae) and their hybrid progeny. Personal communication.
- LETCHAMO, W., 1992: Ökologische, genetische und ontogenetische Einflüsse auf Wachstum, Ertrag und Wirkstoffgehalt von diploiden und tetraploiden Kamillen, *Chamomilla recutita* (L.), RAUSCHERT. Dissertation, Justus-Liebig-Universität Gießen.
- LÖSSL, A., 1999: Die cytoplasmatisch männliche Sterilität und CMS aus *Helianthus petiolaris* bei der Sonnenblume. *Cytoplasm Genome Research*, <http://www.lossl.de/index.htm>.
- MADER, E., 1998: Experimentelle Überprüfung des Hardy-Weinberg Gleichgewichts von Bisabolol-Chemotypen bei tetraploiden Kamillen. Diplomarbeit, Veterinärmedizinische Universität Wien.
- MASSOUD, H., 1988: Quantitative Vererbung einiger Ertragsmerkmale und der Hauptkomponenten des ätherischen Öls von Kamille, *Matricaria chamomilla* L. (syn. *Chamomilla recutita* L.). Dissertation, Technische Universität München.
- MATSUMARA, A., NOMIZU, T., FUTURANI, N., HAYASHI, K., MINAMIYAMA, Y., HASE, Y., 2010: Ray florets color and shape mutants induced by 12 C 5+ ion beam irradiation in chrysanthemum. *Sci. hortic.* 123, 558-561.
- ODENBACH, W., 1997: Biologische Grundlagen der Pflanzenzüchtung. Parey Buchverlag, Berlin.
- PANK, F., VENDER, C., VAN NIEKERK, L., JUNGHANNS, W., LANGBEHN, J., BLÜTHNER W.-D., NOVAK, J., FRANZ, C., 2001: Combining ability of *Origanum majorana* L. Strains – agronomical traits and essential oil content: results of the field experiment series in 1999. *Journal of Herbs, Spices and medicinal Plants* 9, 31-37.
- SINGH, R., 1992: Chromosomal abnormalities and fertility in induced auto-tetraploid *Helianthus annuus* in C₁ and C₂ generation. *Cytologia* 57, 277-281.
- VETTER, S., FRANZ, C., 1998: Samenbildung bei Kreuzungen und Selbstungen mit polyploiden *Achillea*-Arten (Asteraceae). *Z. Arzn. Gew.pfl.*, 11-14.
- WANG, Y., JHA, A.K., CHEN, R., DOONAN, J.H., YANG, M., 2010: Polyploidy-associated genomic instability in *Arabidopsis thaliana*. *Genesis* 48, 254-263.

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