The Influence of Flower Structures on the Seeds Productivity of the Carrot Breeding Lines

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Summary
The aim of the study was to examine the morphological diversity of flowers of male-sterile (CMS) and male-fertile breeding lines of carrot and its influence on seed setting.

In examined male-sterile carrot population stamens are transformed into petal-like structures of different shapes. It was observed that in some anther-like structures sporogenic tissue degenerates. It is supposed that decrease of seeds productivity of carrot breeding lines could be caused by morphological abnormalities of mother and father-parental flowers. Degeneration of pollen, the absence of endothecium, which is responsible for opening the anthers and growth of pollen tubes on the external area of the pistil, decreasing the number of functional pollen influence negatively fertilization. Moreover, other specific traits of male-sterile mother plants, as reduction of nectaries size, pistils deformation, ovule and embryo degeneration also inhibit process of seed setting. The lowest amount of seeds produced plants with multiple stigma pistils and nectaries deformations. Male-sterile plants with white flower petals, big nectaries, exposed, doubled pistils and big ovaries produced more seeds than the other mother plants.

key words: carrot, flower morphology, petaloid-type CMS, seeds

INTRODUCTION
For many years plants with cytoplasmatic male-sterility trait (CMS) have been used as a mother form in F1 hybrid breeding, including carrot (Banga et al. 1964, Erickson et al. 1982, Nothnagel et al. 2000). The absence of anthers excludes pollen production and it is direct reason why the type of male-sterility where anthers transform into petals (petaloid sterility) is safer and more often used by breeders than brown-anther sterility (Chadha & Frese 1981, Morelock et al. 1996). However, among the individual breeding lines antherless plants with low seeds productivity were observed and in spite of the presence of beneficial traits, they could not be used in a breeding (Kozik & Nowak 2002, 2003).

The aim of this work is to find relation between carrot flowers morphology and its seeds production.
capacity in our own breeding lines used in carrot breeding.

MATERIALS AND METHODS

The experimental work was conducted in years 2006-2008 in the greenhouse of Research Institute of Vegetable Crops in Skierniewice. The three pairs of each parental carrot lines derived from our breeding works were studied. Plants were grown in separate insulators and pollinate by domestic flies. Opened flowers from the main umbel and umbels of the first to the fourth order of parental plants were investigated. Parental plants were represented by five male-sterile (mother) breeding lines and five male-fertile (father) breeding lines. The morphological abnormalities of all examined lines were analyzed on 50-100 flowers. The following flower traits were considered: colour, shape and size of particular flower elements, morphology of ovaries and anthers, pollen germination on stigma and penetration of pollen tubes into ovules. Seeds from each plant were collected from individual umbels. The number of seeds from three plants per line was counted. Morphological and histological study was made with the aid of stereoscopic microscope Olympus SZX16 and Nikon Eclipse 80i microscope with digital system for picturing. Anatomical preparations were made using paraffin method according to Gerlach (1972). Flowers were fixed with CrAF (chromic acid, acetic acid, formalin), dehydrated in ethanol and prepared for microscope analysis embedding in paraffin, cutting and staining with safranine and light green. By the use of scanning electron microscope Jeol JSEM-S1 the surface of stigma was observed. That part of work was carried out in Laboratory of Electron Microscopic in Nencki Institute of Experimental Biology. The growth of pollen tubes was observed in UV light at 24, 48 and 72 h after pollination. Styles used in that analyses were stained with aniline blue and then macerated (Dyki 1978).

RESULTS AND DISCUSSION

White colour of flowers and structure characterized by two big nectaries, doubled style and green ovary with ribbed surface, covered with many hairs predominated in inflorescences of male-sterile and male-fertile plants (Fig. 1,2,4,6). Typical flowers of male-sterile plants contained five anthers with long, stiff filaments (Fig. 1,2). Eisa and Wallace (1969) suggested that a colour of petals depends on the breeding line, which is in the opposition to Nothnagel (1992). The populations obtained in our study showed anthocyanin on the leaf-stalks, similarly as in the wild forms. Some inflorescences developed a single purple florets containing complete petaloid and spoon-shaped petaloids. However, several lines, especially of male-sterile plants had purple-pink petals and ovules (Fig.5). Also, not typical plants with domination of green colour flowers were found (Fig.7). Most frequently flowers were greenish in the midrib and whitish in the distinct regions. Green coloured petals were also noted. Flower with green petaloid corolla had usually normal pistils which were not only
very small but hollowed into petals whorl as well. Furthermore, its styles were very short decreasing their attractions for insects. Kozik & Nowak (2002, 2003) and Dyki et al. (2007) reported that selection of carrot plants with flowers possessing positive traits and diversity of colour and morphology increased possibility to select the most attractive flowers for pollinators. Flower abnormalities observed in pairs of hybridized partners could be also a cause of pollination and fertilization disorders, which finally result in lower number of seeds (Fig. 2,3,7).

It was also noted that in many father plants the number of anthers was reduced to three, two or even one (Table 1, Fig.2). Some of those flowers were characterized by style and ovary deformations as well (Table 1, Fig.3). Anatomical study of male-fertile flowers indicates that reason of abnormalities may be complex. Degeneration of stamen filaments probably inhibits function of vascular system, what could cause inhibition of pollen development (Fig. 2). Besides, the absence of endothecium layer, which is always responsible for opening pollen sacs inhibits releasing pollen grains (Fig. 10,13).

Consequently, it leads to necrosis of internal anthers tissue (Fig. 11,12). Morphological deformations concern petals, styles and nectaries (Fig. 3). According to Thompson’s report (1961) partially fertile plants have been also found. Thus, even if the umbels of the first three orders were sterile, reversion to fertility in the subsequent orders could be observed. It seems to be similar as described by Banga et al. (1964), Chadha & Frese (1981) or Struckmeyer & Simon (1986). That is the reason why anther-like structures in the flowers with distinctive filaments required special, penetrating microscopic observations and staining with aceto-orcein, which enable verification of the presence of sporogenic tissue (Fig. 4,14). Flowers with reduced ovaries and having more than two styles are regarded in this research as deformations (Table 1, Fig. 6,7,8,9). Finally, destructive changes of ovary tissues, which were observed in both mother and father plants, may induce reduction of the number of seeds (Table 1). A number of petaloid flowers were also observed. Some of them were already characterized by researchers, other were fundamentally different or only resembled types, which had already been described.

The following petaloid stamen types were observed: complete petaloid in which all stamens were replaced by petal-like organs, spoon-shaped structures, in which stamens are incompletely transformed into petal-like structures and a petaloid flowers with filamentous structure. Nothnagel et al. (1997) also found flowers with stamens replaced by ‘filament’ rudiments, where petals were small and poorly white. Phenotype reported by Eisa and Wallace (1969) had no bract-like structures.
Fig. 1. Carrot flower of parental plant with open anthers
Fig. 2. Carrot flower of parental plant with active and deformed stamen
Fig. 3. Deformed carrot flower with not opened anthers
Fig. 4-6. Carrot flowers represent three breeding lines without stamens, with different colors of petals and well-developed nectaries:
Fig. 4. The flower with stamen-like petals (typical petaloid form) and reduced, single pistil,
Fig. 5. There flowers with typical for carrots two-parted pistil,
Fig. 6. The flower with three-parted pistil
Fig. 7-9. Carrot flowers represents three petaloid lines:
Fig. 7. The flower without ovary,
Fig. 8. The flower with well-developed ovary and two-parted pistil,
Fig. 9. The flower with well-developed ovary and six-parted pistil.

Fig. 10-13. The transverse sections of parental plant anthers:
Fig.10. The open anther with endothecium,
Fig.11. The anther with endothecium and part of degenerated pollen grains,
Fig.12. The anther with endothecium and with symptoms of pollen degeneration of all sacs,
Fig.13. The anther without endothecium and not- opening pollen sacs. (x 180)
Fig. 14. Anther-like structure stained acetoorcein with visible sporogenic tissue (st).
(x 60)
Fig. 15. Pollen tubes in pistil - pollination and fertilization in UV light microscope (x120)

Fig. 16. Pistils with pollen tube on the surface of stigma. [SEM] (x180)

Fig. 17. Longitudinal sections of plant ovary with setting seeds (x100)

Fig. 18. Longitudinal sections of plant ovary with degenerated ovules (x100)
Table 1. Morphological analysis of flowers and the number of seeds in carrot breeding lines (in the years 2006-2008)

<table>
<thead>
<tr>
<th>Line</th>
<th>Anthers</th>
<th>Pistils</th>
<th>Ovules</th>
<th>Nectaries</th>
<th>The number of seeds per plant</th>
<th>Flowers with deformations %</th>
</tr>
</thead>
<tbody>
<tr>
<td>G 255</td>
<td>0, als</td>
<td>2-3, df</td>
<td>0, s, m</td>
<td>0, s, df</td>
<td>88</td>
<td>40</td>
</tr>
<tr>
<td>M 151</td>
<td>0-5, df</td>
<td>1-3, df</td>
<td>s, m, df</td>
<td>0, s, df</td>
<td>524</td>
<td>32</td>
</tr>
<tr>
<td>G 552</td>
<td>0, als</td>
<td>2-5</td>
<td>m, b, df</td>
<td>0, s, m, df</td>
<td>181</td>
<td>37</td>
</tr>
<tr>
<td>M 765</td>
<td>3-5, df</td>
<td>1-3, df</td>
<td>m, b</td>
<td>s, m</td>
<td>989</td>
<td>30</td>
</tr>
<tr>
<td>G 603</td>
<td>0-5</td>
<td>2-6, df</td>
<td>m, b</td>
<td>b, df</td>
<td>238</td>
<td>42</td>
</tr>
<tr>
<td>M 798</td>
<td>0-5, df</td>
<td>2-5</td>
<td>s, m, df</td>
<td>0, s, df</td>
<td>260</td>
<td>59</td>
</tr>
<tr>
<td>G 413</td>
<td>0, als</td>
<td>2-6, df</td>
<td>s, m, df</td>
<td>s, m, b</td>
<td>264</td>
<td>38</td>
</tr>
<tr>
<td>M 600</td>
<td>5</td>
<td>2-3, df</td>
<td>m, b</td>
<td>m, b</td>
<td>1835</td>
<td>12</td>
</tr>
<tr>
<td>G 378</td>
<td>0, als</td>
<td>2-8, df</td>
<td>s, m, b</td>
<td>s, m, df</td>
<td>3655</td>
<td>14</td>
</tr>
<tr>
<td>M 549</td>
<td>3-5, df</td>
<td>2-3</td>
<td>m, b</td>
<td>b</td>
<td>2125</td>
<td>9</td>
</tr>
</tbody>
</table>

Line: G - mother lines (male-sterile), M - father lines (male-fertile)
Anthers: 0 - no anthers, 3-5 - three to five anthers, als - anther-like structures, df - deformations
Pistils: 1-8 - number of pistils in flower, df - deformations
Ovules: 0 - no ovules, s - small-sized, m - middle-sized, b - big-sized, df - deformations
Nectaries: 0 - no nectarines, s - small-sized, m - middle-sized, b - big-sized, df - deformations

One of petaloid type was named anther type (Chadha & Frese 1981), but it had not been described in details. It can be define as having flowers with spoon-shaped or filamentous petals. Chadha & Frese (1981), Eisa & Wallace (1969), Linke et al. (1999) reported about complete petaloid and Eisa & Wallace (1969), Chadha & Frese (1981), Nothnagel et al. (1997), Wolyn & Chahal (1998), Linke et al. (1999) petaloid with spoon-like structures. The stamens replaced by petal-like organs in complete petaloid had normal structures but were smaller in size (Thompson 1961, Eisa & Wallace 1969) or less ovate but resembling normal petals (Linke et al. 1999).

Differences in the average amount of seeds per plant were associated with the amount of flowers representing individual line (Table 1). However, pollen ability to germinate on the style surface of mother components depends on receptivity of stigmas. In the consequence, sometimes the lack of pollen tubes growth inside styles were not observed after pollination. This could be explained by not favourable thermal and humidity conditions. Dyki & Hoser-Krause (1990) suggested that very often plants sensitivity to temperature and humidity differences influences changes of pollen fertility of male-sterile broccolis and cauliflowers.

At abundant pollination it was found that pollen grains germinated not only on stigma but also on the nectaries surfaces. Some parts of pollen grains germinated penetrating internal tissues of styles (Fig. 15). On the contrary there were visible long, drying up pollen tubes on the external surface as well (Fig. 16). Pollination and fertilization decide for seeds settings and disorders of these...
processes led to degeneration of ovules (before fertilization) and embryos (after fertilization), which may explain necrotic tissues inside weakly developed ovary (Fig. 17,18). Deformations of anthers, ovaries, styles and reduction of nectaries size could restrict the number of seeds in both parental components particular but especially in G 255, M151, G 603, M 798 lines (Table 1).

CONCLUSIONS

1. Abnormalities of mother and father flowers influence decrease of seeds production in the carrot breeding lines.
2. Styles deformations modify progress of pollination and fertilization, which could modify the number of seeds.
3. Morphological traits, responsible for reduction of seeds number include: green colour of petals, reduction of nectaries size, style destruction, ovary absence, ovules and embryos degeneration, anthers and pollen degeneration.
4. Carrot plants with white petals, big-sized nectaries, exposed style with spherical stigma may be the most attractive for pollinators and produced the highest number of seeds.

REFERENCES


WPŁYW STRUKTURY KWIASTÓW NA PRODUKTYWNOŚĆ NASION LINII HODOWLANYCH MARCHWI

Streszczenie
Celem pracy było określenie różnorodności budowy kwiatów marchwi męskosterylnych i męskopłodnych linii hodowlanych oraz ocena ich zdolności do zawiązywania nasion.