Brassica regeneration activities carried out in Italy

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Introduction

In Italy *Brassica* germplasm is mainly conserved by the national genebank located in Bari at the Istituto del Germoplasma (IDG), belonging to the National Research Council (CNR), and by five other institutions (three in Sicily, one in Marche and one in Umbria). The majority of *Brassica* germplasm is conserved in Sicily by the University of Catania (Dipartimento di OrtoFloroArboricoltura e Tecnologie Agroalimentari, DOFATA) and by the University of Palermo (Botanical garden (BG) and Dipartimento di Agronomia, Coltivazione Erbacee e Pedologia (ACEP)). The former holds an active collection of about 460 accessions, mainly landraces and wild species, while the latter holds a collection of wild species and the related herbarium.

Regeneration activities are essential to utilize the biological materials conserved in view not only to store them over time but above all to use them for characterization and evaluation activities and for safety-duplication by other institutions or genebanks. Regeneration of *Brassica* species, which are often self-incompatible, requires specific management for controlled pollination, achieved both by spatial isolation in the field or by the use of isolation chambers, in order to maintain the original genepool.

Of about 1500 *Brassica* accessions conserved in Italy by the above-mentioned institutions, only about 7% have been regenerated recently (Table 1). We describe here the regeneration activities carried out at DOFATA to support the activities dealing with characterization and evaluation and provide all the conditions required for exploiting some types and landraces of interest for vegetable diversification. These activities include in particular those carried out at DOFATA in the last two years dealing with specific tasks of projects funded by the Italian Agriculture Ministry and the European Union. The former is a project related to evaluation and to genetic improvement of violet cauliflower landraces whereas the latter is the GEN RES CT99 109-112 project in which DOFATA has contributed to the regeneration of a European *Brassica* core collection.

Table 1. *Brassica* accessions conserved and regenerated in Italian institutions

	No. of accessions		
Institution[1]	**Conserved**	**Regenerated**	**Method[2]**
ACEP	8	8	op
BG	400	-	-
DBVBA	17	3	op
DOFATA	457	48	cp
IDG	629	37	cp
ISPORT	50	18	op
Total	**1561**	**114**	

[1] ACEP = Dipartimento di Agronomia, Coltivazione Erbacee e Pedologia, University of Palermo
BG = Botanical Garden, University of Palermo
DBVBA = Dipartimento di Biologia Vegetale e Biotecnologie Agro-ambientali, University of Perugia
DOFATA = Dipartimento di OrtoFloroArboricoltura e Tecnologie Agroalimentari, University of Catania
IDG = Istituto del Germoplasma, CNR, Bari
ISPORT = Istituto Sperimentale per l'Orticoltura, Monsanpolo del Tronto, Ascoli Piceno

[2] op = open-pollinated; cp =controlled pollination

Methodology

The protocol to regenerate accessions at DOFATA begins by by sowing seeds in cellular trays in a greenhouse and transplanting plantlets (3^{rd}-4^{th} leaf stage) into 10-litre pots filled with a peat/sand substrate (1:1 in volume). Plants are usually grown in the open till they reach the flowering stage and then moved to the isolation chambers either in a cold greenhouse or in the field. In some cases plants are transplanted directly into the field and we then use isolation chambers covered by nets to protect plants. Usually about 40-50 plants per accession are put into each chamber for a variable period of time depending on the variation in the flowering time uniformity of the plants grown for each accession.

As pollinators, flesh flies (*Sarcophaga carnaria*) are used. These are grown at the institute, starting with larvae bought in fishing tackle shops. Each week about 1000-2000 larvae are grown in one-litre containers filled with peat and covered by a net till the adult stage is reached. Metamorphosis occurs when the temperature is between 13°C and 28°C, depending on the season; on average, 70-80% of larvae reach the adult stage. Adult flesh flies are released into the isolation chambers when the plants reach the flowering stage.

During the presence of the flies in the chambers no chemical product is used to control pests and diseases to avoid killing the pollinators. Sometimes a protein-rich commercial product (Bumital®) is sprayed in order to prolong the life of the flies in the chambers, especially when there are few flowering plants to feed them. At the end of the flowering stage the plants are moved from the chambers to the field to complete the fruit ripening stage.

Results

The activities carried out have so far allowed the regeneration of 48 accessions of *B. oleracea* var. *botrytis*, *B. oleracea* var. *acephala* and some *Brassica* wild species widespread in Sicily (Fig. 1).

Fig. 1. Some stages of *Brassica* regeneration activities carried out at DOFATA.

Metamorphosis took place at 8 and 18 days at 28°C and 13°C respectively (Fig. 2). This aspect is very important to synchronize the availability of adult flesh flies with plant flowering in order to regenerate all the plants utilized for each accession at the right time. The mean seed yield obtained per plant, for the accessions regenerated, was 28.4 ± 18.4 g for cauliflower, 21.8 ± 15.5 g for kale and 8.8 ± 2.6 g for some wild *Brassica* species. The protocol used is simpler and more efficient than those based on the use of honey bees (*Apis mellifera*) and bumble bee (*Bombus terrestris*) colonies which are much more expensive and not very suitable for the small isolation chambers we use.

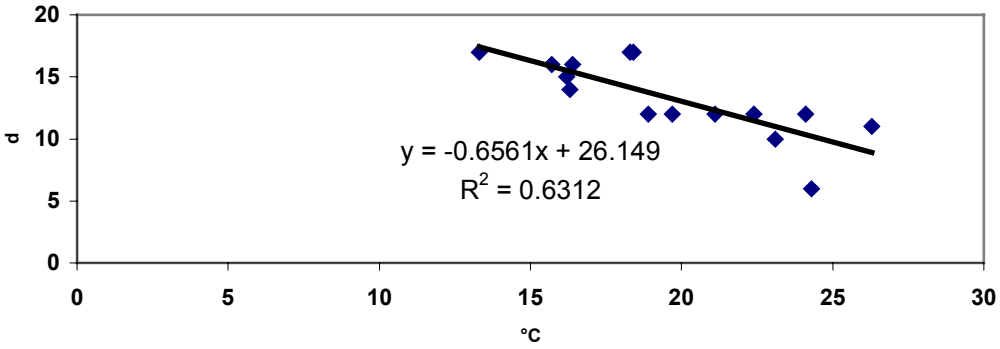

Fig. 2. Correlation between temperature and metamorphosis timing.

References

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