The virome of *Chrysanthemum* at the service of varietal recovery

Restuccia P[1], Miozzi L[2], Marian D[2], Copetta A[3], Pamato M[3], Vinci G[1], Vinotti P[1], Capello M[1], Vaira AM[2]

[1]Florcoop Sanremo, 248 Regione Periane, 18018 Taggia (IM), Italy
[2]IPSP-CNR di Torino, 73 Strada delle Cacce, 10135 Torino, Italy
[3]CREA-OF di Sanremo, 508 Corso Inglesi, 18038 Sanremo (IM), Italy

EUROPEAN UNION

ITALIAN REPUBLIC

LIGURIA REGION

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INTRODUCTION

- In Italy the chrysanthemum market at national level is divided as follows: chrysanthemums grown for 12 months a year (programmed) and single-stemmed uniflora varieties produced and marketed for the All Souls celebration.
- The chrysanthemum is susceptible to virus and viroid infections that can be transmitted by insects, cultural practices, contact with infected plants or by seed.
- For these pathogens there is no cure, only preventive control. The vegetative propagation in chrysanthemum causes the transmission of infections to the new cuttings and leads to pathogens accumulation, reduction of the vigor of the plant and heavy symptomatology in plants, leaves and flowers.

Dilana, Snowdon and Turner varieties of chrysanthemum are still required by the Italian market, however, they have lost the intrinsic positive characteristics of the variety due to the viral diseases spread by the continuous agamic propagation.

DILANA

SNOWDON

TURNER

AIM

- **DIAGNOSIS OF VIRUSES AND VIROIDS -> through Next Generation Sequencing techniques, hunting for known and new viral forms affecting plants and serving for controls in new molecular diagnostic assays for the control of restored plants**

- **RESTORATION OF VARIETIES -> the plants will be placed *in vitro*, heat treated, and the meristems will be collected and micropropagated**

- **VIRUS-FREE CHECK -> analysis of the absence of viruses in the micropropagated material**

- **CHRYSANTHEMUM ACCLIMATIZATION -> The restored plants will be set in an insect-proof greenhouse to obtain a pool of mother plants used for the production of agamic material**

SAMPLES COLLECTION

DILANA variety - red and yellow
Sampled in november 2017 and december 2021
White/chlorotic interveinal areas, redness, chlorosis, leaf curling

TURNER variety – red, white and yellow
Sampled in november 2019 and december 2021
Stunting, leaf deformation, necrosis

SNOWDON variety – white and yellow
Sampled in november 2017 and december 2021 - Mosaic and necrosis, chlorosis, leaf deformation, redness

PIPELINE FOR THE VIROME ANNOTATION AND VARIETY CHECK

Total RNA extraction from symptomatic chrysanthemum plants and varieties to be restored
(Spectrum™ Plant Total RNA Kit)

↓

Pre-processing of NGS reads
(removal of adapters, low-quality and ribosomal reads)

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Annotation of the virome by VirusDetect

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Reconstruction of viral genomes
(VirusDetect, metaspades, EGassembler)

↓

Set up of molecular diagnostic assays for selected viruses

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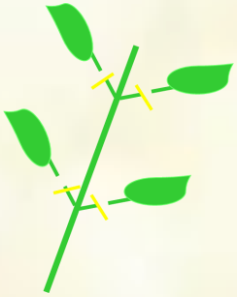
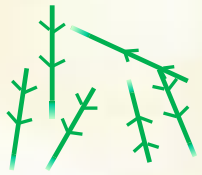
Verification of the absance of viruses and viroids in the restored varieties
(Illumina NGS, molecular diagnosis)

RESTORATION OF VARIETIES



CUTTING OF LEAVES



EtOH 70% x 20'' + NaClO 2% x 20' + 2 rinsed in H_2O x 10'

SUPERFICIAL STERILIZATION

AFTER 2 WEEKS

Murashige Skoog salt and vitamins (MS) substrate

IN VITRO ESTABLISHMENT

MS + BA 1 mg/l + IAA 0.1 mg/l

AFTER 5-6 WEEKS

MULTIPLE SHOOT INDUCTION

MS + BA 0.3 mg/l

THERMOTERAPY AT 37 °C (2 weeks)

MS

SAMPLING AND CULTURE OF MERISTEMS

REGENERATION

VIRUS CHECK AND SHOOT MICROPROPAGATION

- **VIRUS CHECK IN RECOVERED *IN VITRO* PLANTS**
- **DESIGN OF MOLECULAR DIAGNOSTIC TOOLS**
- **ACCLIMATIZATION TEST**

However, a first *in vitro* rooting induction test was performed

MS salt and vitamins without hormones

IN VITRO ROOTING INDUCTION

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