GRAPEVINE VIRUSES (GLRAV-1+GVA) INHIBITS PIGMENTS, RUBPC AND PHOTOSYNTHETIC ACTIVITIES IN FIELD GROWN GRAPEVINE (*VITIS VINIFERA* L.CV. MARZEMINO) LEAVES

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The most common methods to eradicate viruses are thermotherapy *in vivo*, *in vitro* and meristem culture (3). The present work was to investigate the effect of GLRaV-1 and GVA infection mainly on pigments, soluble proteins, ribulose-1,5-bisphosphate carboxylase and photosynthetic activities in field grown grapevine (*Vitis vinifera* L. cv. Marzemino) leaves.

The present study deals with virus elimination in grapevine using *in vitro* thermotherapy. One node-segments of a (GLRaV-1 and GVA) infected clone of 'Marzemino' were surface sterilized, positioned in solidified culture medium and kept in a thermotherapy chamber provided by cool white fluorescent lamps (40 $\mu E\ m^{-2}\ s^{-1}$) with 16/8 h photoperiod at 34°C for 40 days. After this treatment the axillary bud from each green shoot explant was aseptically excised and subcultured on same medium supplemented with 2 mg/l of BA (5). Established daughter vines of original (MP) and heat treated (HT) clone were tested by ELISA for GLFV, ArMV, GLRaV-1, GLRaV-3 and GVA, using a commercial kit (Agritest, Valenzano-Bari, Italy). ELISA tests were carried out on leaves and dormant canes samples of both (MP) and (HT) vines for five years (1998-2002). HT vines resulted GLRaV-1 and GVA free. Both original and heat-treated *ex-vitro* material was propagated on virus-certified rootstocks (Kober 5BB). An experimental vineyard with two blocks was established in the year 2000 at Ala (South Trentino, Italy). The vines are simple pergola system trained. In 2002, samples of 20 leaves from MP and HT plants were gathered at veraison (5 Aug.) and at harvest (16 Sept.). Fresh weight of blades and petioles and length of veins and petioles were measured. Chemical analysis of blades and petioles were carried out (9). Amounts of chlorophyll (Chl), carotenoids (Car) and total soluble proteins were spectrophotometrically determined by two methods (7) (2). The crude leaf extract isolated and assay of ribulose-1,5-bisphosphate carboxylase (RuBPC) activity was measured by Bowes and Ogren (1). All measurements of Chl fluorescence were performed, on detached leaves, with portable PAM-2000 fluorometer (Walz, Effeltrich, Germany). Thylakoid membranes were isolated and reactions of photosynthetic electron transport mediated by whole chain ($H_2O \rightarrow MV$), PSII ($H_2O \rightarrow DCBQ$, $H_2O \rightarrow SiMo$) and PSI ($DCPIPH_2 \rightarrow MV$) were measured as described by Nedunchezhian *et al*. (8). Thylakoid membranes and crude leaf extracts were separated using the polyacrylamide gel system of Laemmli (6).

The contents of Chl and Car were decreased in MP leaves (Fig. 1). This would be due to the grapevine virus, witch probably enhanced the chlorophyllase activity in the leaves. In MP leaves the Car/Chl ratio increased while the Chl *a/b* ratio decrease (Fig. 2). Total soluble proteins and RuBPC activity were reduced markedly in MP leaves (Fig. 3). This relatively low level of soluble proteins might have been due to decrease in the synthesis of RuBPC, the major soluble protein of leaf (Fig. 3). Such reduction in the RuBPC was found due to inhibition of protein synthesis induced by grapevine virus in leaves. This is also supported by SDS-PAGE analysis of crude leaf extracts of RuBPC proteins showed a marginal loss of both large (55 kDa) and small (15 kDa) unit proteins in MP leaves (Fig. 4b). The HT leaves showed a good PSII activity, measured as the Fv/Fm and the Fv/Fm was decreased in MP leaves. The decrease in Fv/Fm ratio was mainly due to decrease in variable fluorescence (Fig. 5). Analysis of various electron transport chain, the PSII and whole chain electron transport markedly inhibited by virus (Fig. 6). Among the various electron donors, DPC and NH_2OH were found to be slightly restoring the PSII activity in MP leaves (Fig. 7). Supporting evidence for the damage to PSII activity was obtained from the thylakoid polypeptide analysis: a comparison of thylakoids of MP leaves with those of the HT leaves showed specific loss of 33, 27-25, 23 and 17 kDa polypeptides (Fig. 4a). Analysis of various mineral contents, not significant changes were observed between HT and MP leaves (Table).

In this work we have studied the influence of grapevine virus (GLRaV-1 + GVA) on some features of the thylakoids from field grown grapevine (cv. Marzemino) leaves. Total chlorophyll, carotenoids, soluble proteins and ribulose-1,5-bisphosphate carboxylase level decreased in virus infected (MP) leaves. In isolated thylakoids, grapevine virus (GLRaV-1+GVA) caused marked inhibition of whole chain and photosystem (PS) II activity. The artificial exogenous electron donors, DPC and NH_2OH marginally restored the PSII activity in MP leaves. The Fv/Fm was also decreased in MP leaves without increasing Fo. The marked loss of PSII activity in MP leaves was evidently due to the loss of 33, 27-25, 23 and 17 kDa polypeptides. The content of various minerals are slightly changed in MP leaves. Thus, our results suggest that the loss of pigments, soluble proteins, RuBPC and photosynthetic activities in infected leaves was due to the virus induced rapid senescence or aging in field grown grapevine leaves.

References

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Fig. 1: contents of Chl and Car in leave

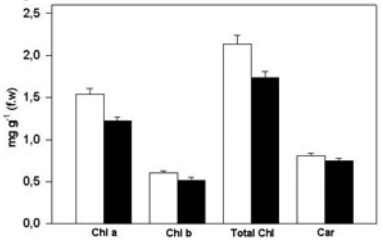

Fig. 2: Chl *a/b* and Car/Chl ratio in leaves

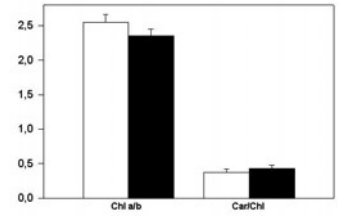

Fig. 3: total soluble protein and RuBPC activity in leaves

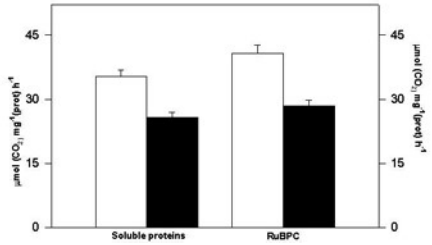

Fig. 4: SDS-PAGE analysis of crude leaf extracts of RuBPC proteins

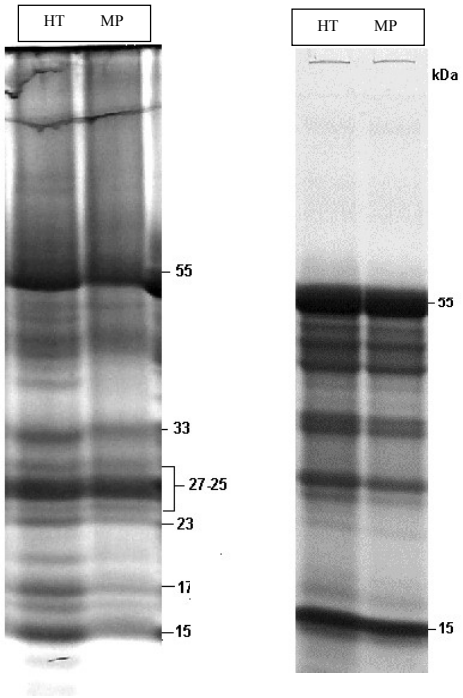

Fig. 5: Chl fluorescence analysis of leaves

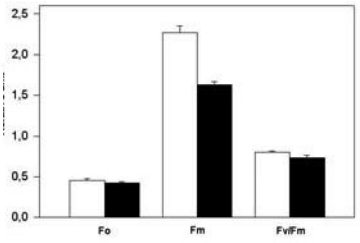

Fig. 6: analysis of various electron transport chain

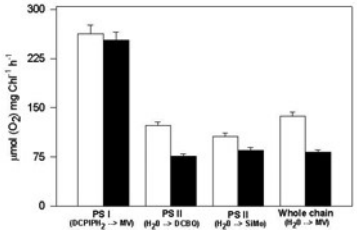

Fig. 7: analysis of various electron transport chain

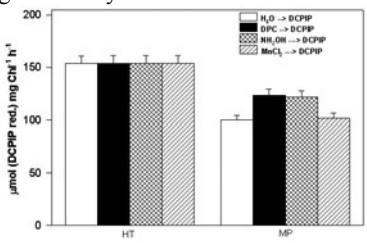

Table Mean values ± std. dev. of some morphological and chemical variable of leaves (blade and petiole).

Characteristics	MP	HT
leaf's weight (g)	5,12 ± 0,09	4,79 ± 0,08
blade's weight (g)	4,23 ± 0,19	3,88 ± 0,09
petiol's weight (g)	0,88 ± 0,09	0,91 ± 0,01
vein's length (mm)	135 ± 4,2	135 ± 0,7
petiol's length (mm)	98 ± 8,4	103 ± 4,2
SPAD index	33,9 ± 0,1 **b**	37,6 ± 0,0 **a**
N blade (% s.s.)	2,24 ± 0,33	2,56 ± 0,11
P blade (% s.s.)	0,28 ± 0,03	0,26 ± 0,06
K blade (% s.s.)	1,65 ± 0,01	1,59 ± 0,06
Ca blade (% s.s.)	2,49 ± 0,32	2,92 ± 0,38
Mg blade (% s.s.)	0,32 ± 0,01	0,32 ± 0,03
Fe blade (% s.s.)	76 ± 7	88 ± 20
Mn blade (% s.s.)	174,5 ± 21,9	194,5 ± 57,3
Bo blade (% s.s.)	26 ± 1,4	27 ± 2,8
Zn blade (% s.s.)	34,5 ± 3,5	39 ± 15,5
Cu blade (% s.s.)	410 ± 209	410 ± 249
S blade (% s.s.)	0,16 ± 0,0	0,20 ± 0,01
N petiol (% s.s.)	0,49 ± 0,03	0,51 ± 0,03
P blade (% s.s.)	0,63 ± 0,12	0,51 ± 0,07
K petiol (% s.s.)	4,78 ± 0,25	4,70 ± 0,08
Ca petiol (% s.s.)	1,92 ± 0,40	2,00 ± 0,44
Mg petiol (% s.s.)	0,62 ± 0,14	0,61 ± 0,15
Fe petiol (% s.s.)	12,5 ± 0,7	13,5 ± 2,1
Mn petiol (% s.s.)	82 ± 7	95 ± 5

Note: different letters of the same variable given significantly differ (p 0.05) by Tukey's test