Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - D.22

ANTHOCYANINS ACCUMULATION AND RELATED GENE EXPRESSION IN RED ORANGE FRUIT INDUCED BY LOW TEMPERATURE STORAGE

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anthocyanins, cold stress, gene expression, real time RT-PCR

Anthocyanins are water soluble pigments belonging to the flavonoids compounds family in nature involved in a wide range of functions; since these pigments impart much of the colour and flavour of fruits and vegetables they are considered components of human diet not exclusively as food products but also as therapeutic agents; in this respect the anthocyanins have been suggested to protect against oxidative stress, coronary heart diseases, certain cancers and other age related diseases (Ross et al., Annu. Rev. Nutrition, 22:19-34, 2002). Nowadays, the anthocyanin's biosynthesis pathway has been almost completely elucidated and most of the structural genes encoding the enzymes responsible for each steps have been isolated from different sources (Holton and Cornish, The Plant Cell, 7:1071-1083, 1995). The activity of the anthocyanins biosynthetic genes is largerly regulated at transcriptional level and consequently the pigmentation pattern must be specified by the expression patterns of the regulatory genes. Moreover, several environmental stimuli can activate the transcription of anthocyanins biosynthetic genes such as light, osmotic and cold temperature stresses (Chalker-Scott, Photochemistry and Photobiology, 70:1-9, 1999). The cold induction of pigmentation has been studied in flower development and related to activation of the expression of anthocyanin biosynthetic genes, including phenylalanine ammonia lyase (PAL), chalcone synthase (CHS), dihydroflavonol 4 reductase (DFR) and anthocyanidin synthase (ANS) (Martin and Gerats, The Plant Cell, 5:1253-1264, 1993). As regards tree fruits, studies on low temperature induced anthocyanins accumulation have been only conducted on apple skin (Reay, Scientia Horticulture, 79: 113-119, 1999) and peach seedlings (Leng and Qi, Scientia Horticulture, 97:27-39, 2003); therefore, the knowledge of low temperature effects in regards to the fruits eatable portions is still missing. In this work, we studied the impact of a low temperature (4°C) during a moderately long storage period (75 days) on sweet orange (*Citrus sinensis* L. Osbeck, cv Tarocco) anthocyanins production and on the expression of structural genes involved in their biosynthesis such as PAL, CHS, DFR and UFGT whose partial cDNA clones have been previously isolated (Lo Piero et al., J. Plant Biochem. Biotech., 14: 1-6, 2005). Moreover, the above mentioned parameters have been also monitored in orange samples in which cold treatment was extended only for 45 days being they subsequently placed at 25 °C for further 30 days. A third oranges group was stored at 25 °C for the entire experimental period representing the control samples. Our results showed that low temperature induced anthocyanins accumulation in sweet orange juice vesicle reaching after 75 days values eight times higher compared with those kept at 25 °C. Besides, real time RT-PCR showed that expression of PAL, CHS, DFR and UFGT was strongly induced during low temperature exposure since levels of all transcripts increased at least 40 fold with respect of control samples. Interestingly, oranges fruits subjected to a brief low temperature exposure (45 days) still maintained higher levels of anthocyanins than those registered in control samples even after a subsequent 30 days storage period at 25 °C, thus suggesting that 45 days exposure was sufficient to get at least a two fold increase. Concordantly, expression of CHS, DFR, and UFGT was always much higher in samples subjected to brief induction than in the control samples. Only PAL transcripts rapidly decreased due to the temperature change 4°C to 25 °C indicating that "early" and

“late” gene implicated in anthocyanins biosynthesis might have been affected by different regulation mechanisms.