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# STRATEGIES FOR THE IMPROVEMENT OF WATER STRESS TOLERANCE OF ACTINIDIA (ACTINIDIA DELICIOSA A. CHEV) AND BIOCHEMICAL MARKER RESEARCH FOR EARLY SELECTION.

Actinidia is one of the species cultivated in the Mediterranean area known for a prominent susceptibility to water deficiency. Irrigation in the most critical phases of the annual cycle plays a fundamentally important role not only in assuring adequate growth of the fruits and, therefore, a good crop yield, but it also influences the plant's productivity in the successive year by preconditioning mineral nutrition and flower induction. A weakening of the plant caused by a water deficiency can also result in it becoming more susceptible to biotic stresses.
Numerous studies have been conducted on the irrigation of actinidia orchards and on the physiology of the species aimed at verifying the crops' behaviour under water stress, which have allowed for the acquisition of useful information for crop improvement.
Diverse species belonging to the Actinidia genus are found in nature, and numerous are the hybrids as well. Natural mutations or spontaneous crosses have given origin to a great variety of different characteristics and, thus, access to a vast genetic base utilisable for the selection of new varieties (*Testolin et al., 2016*). Nonetheless, many interesting agronomical traits, such as cold climate adaptation, disease resistance, including drought tolerance, are not present in the most cultivated variates.

The principal causes which have stood in the way of fully harnessing the genetic potential and have rendered traditional genetic improvement lengthy and costly, have been the dioecious nature of the plant, the fact that the male individuals' genetic background is unknown, in addition to a relatively long juvenile phase.
Furthermore, the different levels of ploidity amongst actinidia species represent another obstacle for the interspecies hybridisation; numerous attempts at producing plantlets through interspecies hybridisation have failed (Pringle 1986*;* Mc Nelage and Considine 1989; Mu *et al* 1990; Ke *et al* 1991).
The genetic transformation techniques represent an extremely advantageous opportunity for the genetic improvement of

woody fruit-bearing plants, including actinidia, which usually are characterised by a lengthy youth period, a high level of heterozygosity and by a low genetic variability. In addition, unlike other technologies applied to date, this technique allows for the targeted "improvement" of certain traits of widely used commercial varieties in a brief amount of time, performing a sort of "gene therapy," without carrying out a profound modification to the plant's DNA, thus maintaining the commercial varieties' peculiar traits. The first transgenic woody plants, with potentially agronomically useful genes, have been obtained precisely in actinidia (*Actinidia deliciosa* A. Chev.) on a late-blooming pollinator, GTH (Giallo Tardivo Hayward) which was transformed with *A. rhizogenes rolABC* genes (Rugini 1989; Rugini *et al.,* 1991). These pioneering works were followed by others, which have lead to the constitution of female actinidia plants of the cv. Hayward some transgenic for *rol* genes and others for *osmotin* (Rugini *et al.* 2000) which were the object of in-field experimentations up to the year 2012 hosted by the Research University of Tuscia (Rugini, 2012).

## Materials and Methods

**The in-field observations,** on 11 years old plants, were focused on foliage water loss in the following genotypes:

1) male plants GTH (Giallo Tardivo Hayward) modified with the *rol ABC* from *A. rhizogenes* using the bacteria's natural promoter;
2) Cv Hayward variety plants modified with *rolABC*, *rolB, rolC* genes through the bacteria's natural promoter;
3) plants grafted with reciprocal grafts between GTH *rolABC* and GTH wt;
4) Cv Hayward variety plants modified with the tobacco *osmotin* gene under the control of the constitutive promoter *CaMV35S* (Rugini, 2012; Rugini 2015);
5) Control plants (wt) of both GTH and Haward;
6) Hayward plants regenerated from callus (regenerated Hayward wt).

Furthermore, analyses were performed relative to certain physical parameters of the terrain on soil samples collected in the experimental area at 40 cm of depth, immediately following those performed on the foliage water loss, namely: granulometric evaluation (sand, lime and clay according to the dimensional limits of the I.S.S.S., with the wet sieving and sedimentation method or pipette method) and the water retention curve including the calculation of the hydrological constants of the field water capacity (-33kPa) and the point of permanent wilting (-1500 kPa ) using the method of the Richards' pressure plate apparatus -).

**The *in vitro* and biochemical analyses** were performed upon two transgenic genotypes of actinidia: Hay *osm1* and Hay *osm166*, derived from two different transformation events and compared with the respective controls: Hayward *wt* and a genotype of Hayward *wt* regenerated from callus which had stood out in field for its tolerance to drought. The shoots were multiplied on a solid substrate for multiplication composed of salts and MS vitamins (Murashige and Skoog, 1962), with added BAP (0,7 $mgL^{-1}$ ), NAA (0,01 $mgL^{-1}$) and Plant Agar 0,6%, pH 5.8, enriched with various concentrations (0, 1, 2, 3%) of polyethylene glycol (PEG), before sterilising in autoclave at 121˚C for 20 minutes. The tests were conducted in pots with 250 mL capacity with 50 mL of substrate. 3 pots containing 10 shoots each were used for each of the various PEG concentrations.

The evaluations, conducted after 4 weeks of growth in a grow room at 23˚C, evaluated the count of newly formed shoots and the relative number of nodes per shoot, besides the weight of the basal callus. A portion of the plant material (shoot without basal callus) was preserved at -80˚C for biochemical analyses that, in particular, focused on the calculation of proline concentration, total protein, thiolic compounds, and of malondialdehyde (MDA) and the variation of the activity of certain stress-related enzymes related to water stress (guaiacol peroxidase and catalase), utilising techniques previously described by Bradford (1976), Santangelo *et al.* (2003) and Astolfi *et al.* (2005). The principal scope was the identification of enzymatic and non-enzymatic markers capable of precocious selection among a multitude of genotypes of the ones most tolerant to water stresses. Specifically, the objective was to check for possible differences in the water stress response mechanisms activated in plants transformed with osmotin and in control plants regenerated from callus (regenerated Hayward wt).

## Results and discussions

### Field tests

The granulometric evaluation of the soil upon which the plants subject of the work persisted allowed for its classification as "loamy" according to the relative triangle of the I.S.S.S. texture. The knowledge of the water retention curve, specifically for the soil involved in the

experimental tests was useful for a comparative valuation including the gravimetric humidity content found in the soil at the moment of sampling. In the experimental area the average humidity, in the period of sampling, dropped below the point of permanent wilting, as can be deduced in comparing it with the water retention curve (*Table 1*). The plants transformed with the *rol* genes exhibited the notable phenotypic traits conferred by these genes and already described in previous works (Rugini *et al.,* 1997 Gutiérrez-Pesce and Rugini 2008; Rugini 2012 and 2015) in addition to a better tolerance to water stress, due probably to the improved efficacy of the foliar apparatus. This affirmation finds a confirmation in the fact that, among the grafted plants, only transgenic (genes *rolABC* and *rolB*) grafts resulted tolerant to drought, while the reciprocal ones did not (*Table 2*). Hayward *rolB* plants, which, beyond their tolerance to water stress, also presented fruits of analogous dimensions to those of the mother plant (Rugini 2012 and 2015) are of particular interest from an agronomic and commercial point of view. The plants transgenic for the *osmotin* exhibited a regular flowering and fruit production analogous to the control, without showing apparent morphological differences, including the sizing and shape (Rugini *et al.,* 1998; Rugini, 2012); the fruits exhibited a clear tolerance to *Botritis cinerea* (botrytis) and to *Cadophora luteo-olivacea* (cadophora) as reported in previous works (Rugini *et al.,* 2011). Similarly, the leaves presented the same property, verified with artificial infections with botrytis mycelium in agar disks (*Figure 1*). Likewise to the *rolABC* and *rolB* plants, they showed a tolerance to water stress, deducible from the state of wilting observed in comparison to other control plants in soil humidity conditions nearing the point of wilting (*Table 2*). Among the non-transgenic plants a somaclone of particular interest (regenerated Hayward wt), produced from the callus of Haward wt, which showed a nearly similar tolerance in field to the one of transgenic plants for osmotin and *rol* genes.

**Table 1** Granulometric composition and hydrological components of the soil (±DS) at the moment of sampling for the hydric state of the leaves of 11 year old Actinidia plants in the summer period (end of June 2012).

| Granulometry (I.S.S.S.) | % clay 50,53 | %lime 23,78 | %sand 25,69 |
|---|---|---|---|
| Field capacity (-0.33 kPa) (Humidity % in weight) | | 38,08. ± 0,77 | |
| Point of wilting (-1500 kPa) (Humidity % in weight) | | 24,94 ± 0,18 | |
| Gravimetric humidity % (Humidity % in weight) | | 23,63 ± 1,95 | |

**Table 2**
Assessment of the state of foliage wilting of the different self-rooted or grafted genotypes present in the field in absence of irrigation. (A = Wilted; VA = early wilting; V = Not wilted), at the moment of explantation (end of June, 2012, starting point of wilting irreversible).

| THESIS | STATE OF FOLIAGE WILTING |
|---|---|
| HAY wt | A |
| Regenerated HAY wt | V |
| HAY Osmotin (various clones) | V-VA |
| GTH wt | A |
| GTH rolABC | V |
| HAY rol ABC | V |
| HAY rol B | V |
| GTH rol ABC / GTH wt | V |
| GTH wt / GTH wt | VA |
| GTH wt / GTH rol ABC | VA |
| HAY wt / HAY wt | A |
| HAY wt / GTH rol ABC | A |

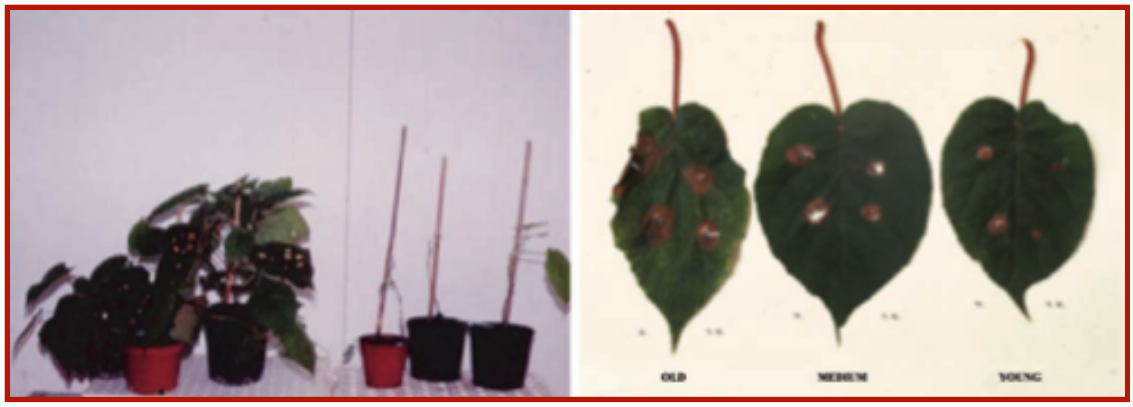


**Figure 1** Artificial infections with *Botritis cinerea* on leaves and fruits with an agar tile containing mycelium of the fungus, performed on all the leaves (old, mature, young). Note how the control plants have lost all their leaves (right) following treatment with the fungus, while the transgenic ones (left) have limited the sickness to the surface occupied by the tile.

## Laboratory tests

The tests conducted in the laboratory evaluated the response *in vitro* to water stress induced in shoots of two transgenic osmotin kiwi genotypes, derived from two distinct transformation events in contrast to the Hayward wt and regenerated Hayward wt controls. The transformed shoots showed a superior tolerance to PEG-induced water stress compared to the Hayward wt control and, quite similarly to the new stress-tolerant genotype, regenerated Hayward wt (*Table 3*).

**Table 3** Growth parameters (Average number of shoots, number of nodes, weight of the basal callus) of the Hayward wt clones (control), regenerated Hayward wt, Hayward osm1 and Hayward osm166. The averages, which are indicated with a different letter for each group of genotypes, show the significant differences over p≤0,05 (Tukey Test).

| Clone | PEG 8000 (% w/v) | Shoot Number | Number of Nodes | Basal Callus |
|---|---|---|---|---|
| Hayward *wt* | 0 | 1,67 a | 5, 11 a | 0,25 a |
| Hayward *wt* | 1 | 1,56 a | 3, 67 b | 0,14 b |
| Hayward *wt* | 2 | 1,68 a | 3,88 b | 0,10 b |
| Hayward *wt* | 3 | 1,75 a | 3,77 b | 0,16 b |
| | | | | |
| regenerated Hayward *wt* | 0 | 2,22 a | 7,11 a | 0,52 a |
| regenerated Hayward *wt* | 1 | 1,89 a | 6,01 ab | 0,25 b |
| regenerated Hayward *wt* | 2 | 2,01 a | 5,01 b | 0,29 b |
| regenerated Hayward *wt* | 3 | 1,61 a | 5,55 b | 0,20 b |
| | | | | |
| Hayward *osm 1* | 0 | 1,68 a | 5,22 a | 0,46 a |
| Hayward *osm 1* | 1 | 1,70 a | 5,00 a | 0,29 b |
| Hayward *osm 1* | 2 | 1,77 a | 5,03 a | 0,16 b |
| Hayward *osm 1* | 3 | 1,65 a | 4,89 a | 0,16 b |
| | | | | |
| Hayward *osm166* | 0 | 1,84 a | 6,33 a | 0,36 a |
| Hayward *osm166* | 1 | 1,81 a | 6,70 a | 0,23 ab |
| Hayward *osm166* | 2 | 1,72 a | 5,44 a | 0,12 b |
| Hayward *osm166* | 3 | 1,76 a | 5,67 a | 0,14ab |

The growth of the cultivated shoots showed the same parameters even at the highest concentration of PEG (3%) and similarity to those placed in the control substrate lacking PEG. To the contrary, the growth of shoots of the cv Hayward wt was progressively reduced with the increase of PEG concentration and the leaves were visibly damaged. The regenerated Hayward wt genotype showed a superior growth performance compared to cv Hayward wt, both in terms of number of shoots and nodes as well as basal callus (statistical calculation not shown in table 2). This genotype possessed a major vigour *in vitro* and in field (data not shown). Considering this is a case of regeneration, and, therefore, of a plant produced from competent cells, possibly with different epigenetic traits, it may have acquired a reinvigoration, similarly, but to a minor extent, observed in many genotypes derived from crosses. A tentative hypothesis may be advanced that this phenomenon grows more evident the more the cultivar ages, in other words the number of times agamic multiplication is performed on it increases, unless additional modifications take place due to somaclonal variability. This behaviour has also been observed in the first olive plants of the cv Canino regenerated through somatic embryogenesis from adult maternal tissues. These plants, in fact, exhibited a major youthfulness and vigour in respect to those propagated by stimulation of the axil buds or by cutting (Rugini personal communication).

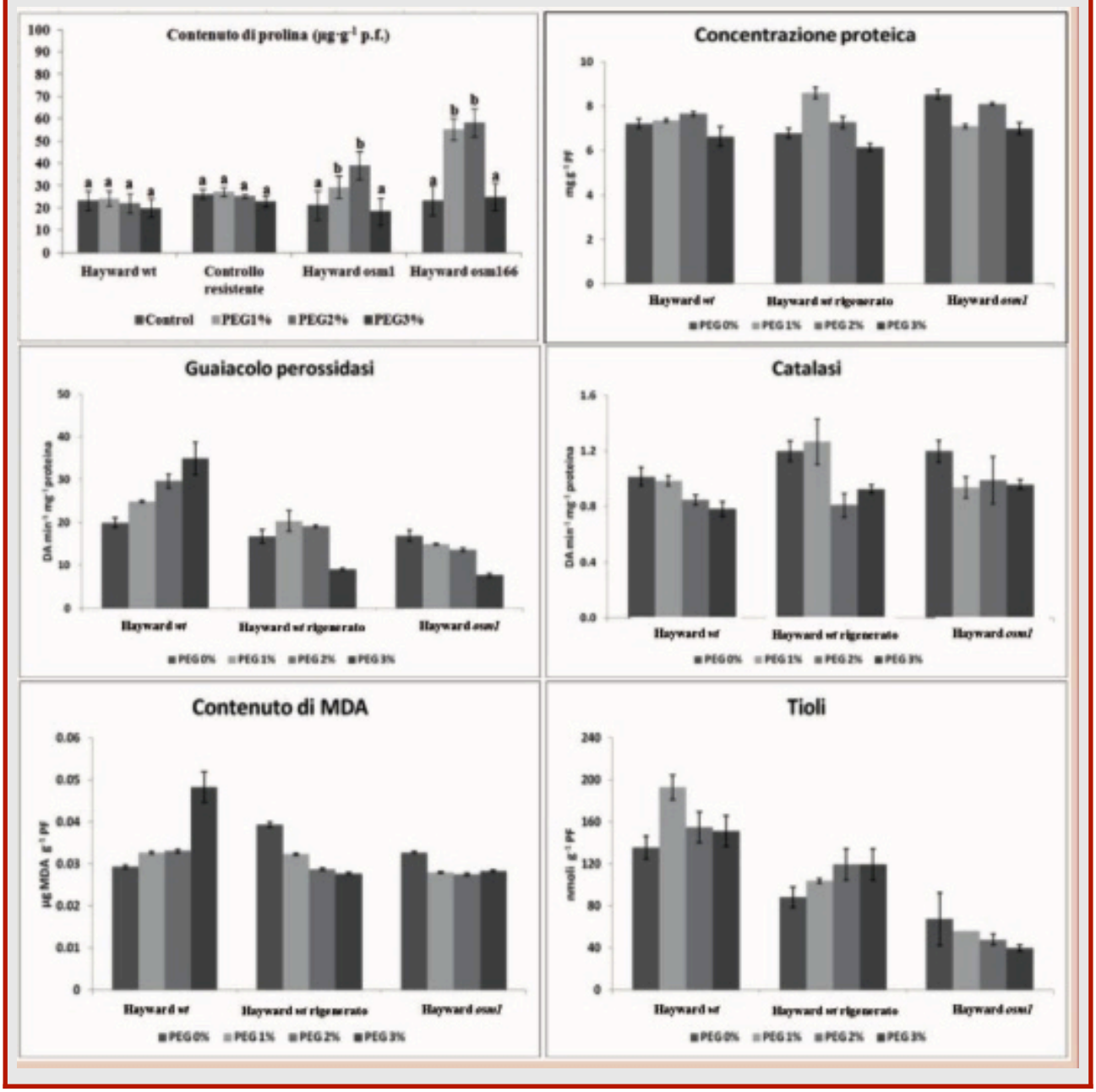
The analysis of the measured biochemical parameters confirmed that the tolerance to induced stress is accompanied by changes at the level of enzymatic activity and of the accumulation of metabolites involved in stress mechanisms (*Figure 3*).

In plants subjected to water stress, a reduction of the photosynthetic activity is generally confirmed, which causes a lower CO2 assimilation rate and the accumulation of reducing agent potency. The latter event determines the increase of reactive oxygen species (ROS) and of free radicals (Schwanz and Polle, 2001; Vranovà et al., 2002); such stress conditions require, therefore, a increase in antioxidant enzyme activity. The plants contain, additionally, numerous non-enzymatic antioxidant compounds, which can detoxify the ROS and, thus, diminish the negative effect of the stress.

In this work, we evaluated the variation in the activity of two antioxidant enzymes - catalase and peroxidase, which are involved in the elimination of $H_2O_2$ from the chloroplasts, and the concentration of the non-proteic thiols as a non-enzymatic marker, was also analysed. On the other hand, we analysed the content of MDA as a indicator of the oxidative stress damage, which allows for the valuation of cellular membrane lipid peroxidation levels. The results seem to indicate that the transgenic genotype plants could be capable of confronting water stress and suffering fewer damages, as indicated by the lower levels of MDA compared to the *wt.* However, in this type of response, there appears to be no implication of either peroxidase or catalase, which do not show relevant variations in the same genotype, following subjection to stress. A different discussion may be had over the regenerated drought-tolerant genotype, which shows, like the transgenic one, lower levels of MDA, but, unlike the transgenic one, shows perceivable variations of the two measured anti-oxidative enzymes, which could explain these plants' better capacity of response to water stress generated by exposure to PEG. After all, the control genotype is subjected to oxidative stress damage (increase of MDA levels following PEG treatment) to which it responds with an increase of antioxidant enzyme activity, principally of peroxidase. The fact that the peroxidase activity increases in Hayward genotype wt tissues during the progressive exposure to water stress, while remaining constant and even decreasing in the transgenic genotype, can be in part explained considering that the peroxidases participate in the modulation of the cell wall properties during plant growth (Bacon *et al., 1997*) and, therefore, a increment in the activity of these enzymes may reflect variations of the mechanical properties of the cell wall, which, in turn, could be correlated to an adaptation to water stress, which would be necessary in the Hayward wt genotype but not in the transgenic genotype (inasmuch as it appears not to suffer damages). Therefore, even in the *wt* genotype, the peroxidase enzyme would not have only a antioxidant role. In order to explain the better performance of the transgenic genotype in terms of water stress, we evaluated the concentration of thiol compounds in the leaves of these plants to verify whether the lack of damage was due to a greater production of these compounds for the neutralisation of the ROS.

The thiols, similarly to cysteine and glutathione, are organic sulphur traces abundantly available in plant tissues and, in normal conditions, the glutathione is the predominant trace.

**Figure 3** Average content of proline (express in µg•g$^{-1}$ of fresh weight), proteic concentration (mg g$^{-1}$ f.w.), guaiacol peroxidase (∂A min$^{-1}$ mg$^{-1}$ proteins), catalase (∂A min$^{-1}$ mg$^{-1}$ proteins), MDA content (µg g$^{-1}$f.w.), of thiols (mmol g$^{-1}$ f.w.) of Hayward wt (control), regenerated Hayward wt, Hayward *osm1* subject to various concentrations of PEG used. Different letters on the graphic are used to indicate significant differences per P<0.01 (Tukey test).




Still, against all expectations, the thiol concentration was augmented in the *wt* genotypes and in the resistant, but not in the transgenic.

The significance of such results and, overall, the interpretation of the behaviour of the transgenic genotype, remain an open question and further elucidations will certainly be drawn from the study of the activity of other antioxidant enzymes, such as superoxidedismutase (SOD) and ascorbic peroxidase (APX), which are key enzymes in the anti-oxidative system of not only chloroplasts, but also other cellular compartments. Furthermore, the measuring of GSH content variations (which could be masked on the inside of the total thiol fraction) could explain the defence mechanism through which the plant operates to reduce the effects of water stress.

In conclusion, from the preliminary results obtained in this work of comparing osmotin transgenic plants to the somaclone — both of which drought tolerant — it may be affirmed that certain physiological parameters, identified in plants transgenic for the osmotin gene, could be employed as possible markers

for the precocious selection of drought-tolerant plants derived through other techniques of genetic improvement. Furthermore, the plants over-expressing osmotin, in addition to representing an opportunity for sustainable agriculture, through the manifestation of greater resistance in terms of biotic and abiotic stresses, could also have important implications for the protection of human health. It is known, in fact, that osmotin belongs to the PR (Pathogen Related) protein family, of the 5th type (Anil Kumar *et al.,* 2015) and, in nature, it is only produced by plants in response to pathogen attack or abiotic stresses of various kinds. It has been observed that osmotin is capable of protecting the chlorophylls and the photosynthesis related metabolic pathways (Barthakur *et al.* 2001; Goes *et al.,* 2010), thus determining the increased proline accumulation, which performs a re-equilibration of osmotic balance and the protection of the cell structure (Subramanyam *et al*., 2011). In terms of human health, of particular importance is the role that osmotin plays in the veterinary and human field of applications, inasmuch as this plant protein is capable of mimicking the activity of the human hormone Adiponectin (Kadowaki *et al.,* 2005).

Adiponectin is a hormone produced by the adipose tissues and is involved in various metabolic processes, such as glucose regulation and the catabolism of fatty acids both in the body and the brain. The scarcity of adiponectin results in diseases such as diabetes, liver conditions and cardiovascular problems (Kadowaki and Yamauchi, 2005; Tang *et al.,* 2005; Holland and Scherer, 2013). For these reasons, today there is a growing interest in the research on osmotin, seen as a protein with a therapeutic potential targeted to humans. Recent publications have demonstrated that osmotin is capable of recovering damages caused by senile dementia, Alzheimer's, diabetes, colitis and Crohn's disease, thanks to its capacity to activate the Adipor1/Sirt1 receptors (Shah *et al*., 2016) and, therefore, to activate the cellular enzymatic cascade improving the functionality of mitochondria and, thus, potentiating the cell's vital activity, including the control of neoplastic growth. A project is underway at the Research University of Tuscia, in Viterbo for the extraction and purification of recombinant osmotin from the transgenic genotype actinidia plants, which, unlike control plants, express the recombinant gene for osmotin from tobacco *Wisonsin 38* with a 35S viral promoter, and, therefore, produce the protein at a factor which could vary between 4% and 16% of the total protein content. These genotypes are, *de facto*, perfect bioreactors capable of supplying osmotin, of great therapeutic worth, in good quantities and in a context of being ready for human and animal consumption, making the commercialisation of this substance economically advantageous for the pharmaceutical industry and plant suppliers alike.

## Bibliography


– ANIL KUMAR, S. HIMA KUMARI, P. SHRAVAN KUMAR, G. MOHANALATHA, C. KAVI KISHOR, P. B. (2015). Osmotin: A plant sentinel and a possible agonist of mammalian adiponectin. Frontiers in Plant Science, 6(MAR).

– ASTOLFI S., ZUCHI S., PASSERA C. (2005) Effect of cadmium on H+ATPase activity of plasma mem- brane vesicles isolated from roots of different S-sup- plied maize (Zea mays L.) plants. Plant Science 169, 361-368. DOI: 10.1016/j.plantsci.2005.03.025

– BARTHAKUR S., BABU V., BANSAL K.C. (2001). Over-expression of osmotin induces proline accu- mulation and confers tolerance to osmotic stress in transgenic tobacco. J. Plant Biochem. Biotech- nol. 10, 31-37.

– BRADFORD MM. (1976) A rapid and sensitive method for the quantitation of microgram quan- tities of protein utilizing the principle of protein– dye binding. Anal Biochem; 72:248–54.

– CANDAN N., TARHAN L. (2012) Tolerance or sensitivity responses of Mentha pulegium to osmotic and waterlogging stress in terms of antio- xidant defense systems and membrane lipid pero- xidation. Environ. Exp. Bot. 75:83–88.

– GOEL D., SINGH A.K., YADAV V., BABBAR S.B., BANSAL K.C. (2010). Overexpression of osmotin gene confers tolerance to salt and drought stres- ses in transgenic tomato (Solanum lycopersicum L.) Protoplasma 245, 133-141.

– GUTIÉRREZ-PESCE P. AND RUGINI, E. 2008, Kiwifruit, in Kole, C. and Hall, T. C. (eds.), "Com- pendium of Transgenic Crop Plants: Transgenic Tropical and Subtropical Fruits and Nuts", Blackwell Publishing, Oxford, UK, pp 185-212.

– HOLLAND W.L., SCHERER P.E. (2013). Ronning after the adiponectin receptors. Science 342, 1460-1461. – KADOWAKI T., YAMAUCHI T. (2005). Adiponectin and adiponectin receptors. Endocr. Rev. 26, 439-451. – MCNEILAGE M.A. AND CONSIDINE J.A. (1989). Chromosome studies

in some Actinidia taxa and implications for breeding. New Zealand Journal of Botany 27, 71-81.
– MU S.K., FRASER L.G. AND HARVEY, C.F. (1990A). Rescue of hybrid embryos of Actinidia species. Scientia Horticulturae 44, 97-106.
– MURASHIGE T, SKOOG F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15:473–479 – PRINGLE J.G. (1986). Potential for interspecific hybridization in the genus Actinidia. New Zealand Agronomical Society Special Publication 5, 365-368. – RUGINI E, CARICATO G., MUGANU M., TARA- TUFOLO C., CAMILLI M. AND CAMMILLI C. (1997). Genetic stability and agronomic evalua- tion of six-year-old transgenic kiwi plants for rolABC and rolB genes In: ISHS Acta Hort. 447 p. 609-610, ISSN 0567-7572
– RUGINI E. (2015) Piante da frutto transgeniche e considerazioni sulle conseguenze dei divieti imposti alla ricerca in Italia. Italus Hortus, Review n. 25, 22 (1): 31-57
– RUGINI E., 2012. Risultati preliminari di una sperimentazione di campo di miglioramento genetico dell'Actinidia con tecniche biotecnologi- che per tolleranza a stress idrico, funghi patogeni e modifica dell'architettura della chioma. Kiwi informa, Luglio/settembre: 4 -18.
– RUGINI E., MUGANU M. GUTIERREZ-PESCE P. (2000). Transformation of Actinidia spp In: In: Molecular Biology of Woody Plants, Vol 2 (S.M. Jain and SC Minocha) eds), Kluwer Academic Publishers, p 191-243.
– RUGINI E., MUGANU M., PILOTTI M., BALESTRA G. M., VARVARO L., MAGRO P., BRESSAN R., TARA- TUFOLO C. (1998). Genetic Stability, transgene he- reditability and agronomic evaluation of transgenic kiwi (Actinidia deliciosa A. Chev.) Plants for rolABC, rol B and Osmotin genes. Forth International Sympo- sium on Kiwifruit Santiago del Chile 11-14 Febr. p 26. – RUGINI E., PELLEGRINESCHI A., JACOBONI A., MARIOTTI D. E MENCUCCINI M., 1989. Trasfor- mazione genetica dell'Actinidia (Actinidia delicio- sa A Chev.) mediante geni "Root inducing" di Agrobacterium rhizogenes. Atti XXXIII Convegno annuale di Genetica Agraria, p 147. Alghero.
– RUGINI E., PELLEGRINESCHI A., MENCUCCINI M. and MARIOTTI, D. (1991). Increase of rooting ability in the woody species Kiwi (Actinidia delicio- sa A. Chev.) by transformation with Agrobacte- rium rhizogenes T-DNA rol genes. Plant Cell Reports, 10:291-295.
– RUGINI E., CRISTOFORI V., MARTIGNONI D., GUTIERREZ-PESCE P., ORLANDI S., BRUNORI E., BIASIR.,MAGROP.,2011.Kiwifruittransgenicsfor osmotin gene and inoculation tests with Botrytis cinerea and Cadophora luteo-olivacea. Acta Hortic. 913:197-204
– SANTANGELO E., FONZO V., ASTOLFI S., ZUCHI S., CACCIA R., MOSCONI P., MAZZUCATO A. AND SORESSI G. P. (2003) The Cf-2/Rcr3esc gene interaction in tomato (Lycopersicon escu- lentum Mill.) induces autonecrosis and triggers biochemical markers of oxidative burst at cellular level. Functional Plant Biology 30, 1117-1125. DOI:10.1071/FP03121

– SCHWANZ, P. AND POLLE, A. 2001. Growth under elevated $CO^2$ ameliorates defenses against photo-oxidative stress in poplar (Populus alba x tremula). Environmental and Experimental Botany 45: 43-53.

– SHAHSA,YOONGH,CHUNGSS.,ABIDMN., KIM T H., LEE H Y., KIM M O (2016) Novel osmotin inhibits SREBP2 via the Adipo R1/AMPK/SIRT1 pathway to improve Alzheimer/'s disease neuropathological deficits. Mol Psychiatry- /03/22/online, Macmillan Publishers Limited. 1 476-5578.

- SUBRAMANYAM K., SAILAJA K.V., SUBRAMANYAM K., RAO D.M., LAKSHMIDEVI K. (2011). Ectopic expression of an osmotin gene leads to enhanced sant tolerance in transgenic chilli peppers (Capsicum annuum L.). Plant Cell Tissue Organ. Cult. 105, 181-192.

– TANG Y.T., HU T., ARTERBURN M., BOYLE B., BRIGHT J.M., EMTAGE P.C., ET AL. (2005). PAQR proteins: a novel membrane receptor family defined by an ancient 7-transmembrane pass motif. J. Mol. Evol. 61,372-380.

– TESTOLIN R., HUANG H.-W., FERGUSON R. (Eds.). (2016). The Kiwifruit Genome. Series Compendium of Plant Genomes, Springer International Publi- shing Switzerland. DOI 10.1007/978-3-319-32274-2 – VERONESE P., RUIZ M.T., COCA M.A., HERNAN- DEZ-LOPEZ A., LEE H., IBEAS J.I. ET AL., (2003). In defense against pathogens. Both plant sentinels and foot

soldiers need to know the enemy. Plant Physiol. 131, 1580-1590.

– VRANOVA E, ATICHARTPONGKUL S, VILLARROEL R, VAN MONTAGU M, INZE D, VAN CAMP W (2002) Comprehensive analysis of gene expression in Ni- cotiana tabacum leaves acclimated to oxidative stress. Proc Natl Acad Sci USA 99: 10870–10875. **Silvestri Cristian, Astolfi Stefania, Celletti Silvia, Cristofori Valerio, Minandri Antonella, Ubertini Giampiero, Ruggiero Bruno e Rugini Eddo**

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