The influence of rootstocks on chilling injury symptoms of 'Big Top' nectarine fruits

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Abstract

The influence of nine different *Prunus* rootstocks budded with the 'Big Top' nectarine cultivar was evaluated in a rootstock trial established at the Experimental Station of Aula Dei (Zaragoza, Spain). Chilling injury (CI) symptoms as browning, mealiness, leatheriness and bleeding were determined after 28 days of cold storage at 5°C plus 2 days of shelf-life. Agronomical basic fruit quality traits as firmness, soluble solids content (SSC) and titratable acidity (TA) were determined at harvest, after 28 days of fruit storage at 5°C and after 28 days plus 2 days of shelf-life. In addition, biochemical compounds as main individual sugars and organic acids, relative antioxidant capacity (RAC), total phenolics content (TPC), total flavonoids content (TFC), vitamin C (AsA) and anthocyanins content (AC) were also quantified. Moreover, enzymatic activity of key enzymes related with the tolerance against CI such as phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO) and peroxidase (POX) were determined. Significant effect of *Prunus* rootstocks was found on leatheriness and bleeding symptoms, firmness, TA, sugars and organic acids, AC and enzymatic activities. Sugars, organic acids, TPC, AC and the enzymatic activities of the phenylpropanoid pathway and ROS metabolism enzymes seemed to be important parameters to consider in the prevention of CI susceptibility. These results may be helpful in the choice of rootstocks inducing lower chilling injury susceptibility.

Keywords: peach, antioxidants, browning, bleeding, enzymes, mealiness, phenols

INTRODUCTION

Nectarines (*Prunus persica* var. nectarine) are a variant of peaches (*P. persica*) which have lack of pubescence in the skin (Lurie and Crisosto, 2005). Nectarines have gradually gained importance in Spain due to the introduction of new full-colored and sweeter nectarines, such as 'Big Top' (Font i Forcada et al., 2019). Peaches and nectarines world production stands to 24.6 million tons where Spain, with a production of 1.3 million tons, is the second largest producer in the world after China (FAOSTAT, 2022). Peach is currently the most important fruit tree species in terms of production and exports in Spain (Iglesias and Echeverría, 2021).

Nectarines and peaches are climacteric fruits that ripen and deteriorate quickly. To increase shelf-life, cold storage is the most common method applied. Nevertheless, chilling injury (CI) symptoms limit conservation (Lurie and Crisosto, 2005).

Rootstocks offer a wide range of possibilities in tree vigor, soil adaptation and fruit quality on the budded cultivars because different species and their hybrids in the *Prunus* genus can be used. A significant effect has already been reported concerning agronomical performance and content of biochemical compounds, such as sugars and antioxidants in fruits (Font i Forcada et al., 2019, 2020; Mestre et al., 2017; Reig et al., 2016). However, the rootstock effect on the postharvest quality of fruit has hardly been explored. Consequently, this study aims to assess the effect of different rootstocks on CI and the biochemical mechanisms involved on the susceptibility to this postharvest disorder. The final objective is to select rootstocks inducing good agronomical performance and fruit quality as well as low CI susceptibility.

MATERIAL AND METHODS

The 'Big Top' cultivar was budded on nine *Prunus* rootstocks with different genetic background: peach-almond hybrids ('Adarcias', 'GF 677' and 'PADAC 9902-01'), a *P. persica* × *P. davidiana* hybrid ('Cadaman'), an hexaploid *P. insititia* plum ('Adesoto 101'), plum × peach-almond hybrids ('PADAC 04-01', 'PADAC 04-03' and 'PADAC 99-05'), and a plum-almond hybrid ('ReplantPAC') in 2007. Trees were established in a randomized block complete design with five replicates per rootstock during the winter of 2008-09.

During the 2021 growing season, 50-60 fruits per each replicate, at commercial harvest maturity (flesh firmness in the range of 40-50 N and fruit color >80% of fruit surface), were taken and analyzed at harvest. Samples were also analyzed after 28 days (28d) of cold storage at 5°C and after 28 days at 5°C plus two days of shelf-life at room temperature (28+2d). For each sampling date, composite flesh samples without skin of ten fruits were taken and frozen in liquid nitrogen, lyophilized and milled to a dry powder. After 28+2d, before quality traits analysis, chilling injury symptoms were evaluated visually according to Lurie and Crisosto (2005) with different numerical scales: browning (1 to 6), bleeding (1 to 6) and mealiness (1 to 3). Leatheriness was evaluated by the ratio of fruits affected from the total (0 to 1).

Flesh firmness (N) was measured using a penetrometer (FT-327). SSC (°Brix) were measured with a digital refractometer (Atago PR-101). TA (mg malic acid g^{-1} dry weight) was determined by titration with NaOH 0.1 M and pH end-point of 8.2 (Metrohm Ion analysis, 807 Dosing Unit). Sugar and organic acids profiles were analyzed by HPLC, using a Rezex™ ROA-Organic Acid H^+ (8%) column (300×7.8 mm, Phenomenex). A photodiode array detector (Waters 2489) at 210 nm and sulfuric acid solution 0.005 N as mobile phase were utilized.

RAC (µg Trolox equivalents g^{-1} DW) was determined according to Brand-Williams et al. (1976) using 2,2-diphenyl-1-picrylhydrazyl (DPPH). TPC (mg of gallic acid equivalents g^{-1} DW) was determined as described by Singleton and Rossi (1965) using the Folin-Ciocalteau reagent. Flavonoids (catechin equivalents g^{-1} DW) were measured by a colorimetric assay (Zhishen et al., 1999). Vitamin C (mg ascorbic acid g^{-1} DW) was determined as described by Okamura (1980). AC (µg cyanidin-3-glucoside equivalents g^{-1} DW) was determined according to the pH-differential method (Giusti and Wrolstad, 2001).

Enzymes and proteins were extracted with Na-phosphate buffer 0.1 M, pH 6.8, according to Galeazzi et al. (1981). Protein content (mg BSA/g DW) was determined using the Bradford method (Bradford, 1976). PAL activity was determined by the conversion of L-phenylalanine to t-cinnamic acid (Tovar et al., 2002). PPO activity was assayed based on the oxidation of 4-methylcathecol (Galeazzi et al., 1981). POX activity was determined by measuring the brown color appearance resulting from the oxidation of guaiacol (Dann and Deverall, 2000). Enzymatic activities were estimated by absorbance measurements at 290 nm (PAL), 420 nm (PPO) and 470 nm (POX) and expressed in units of enzymatic activity (U) per g protein.

Means were statistically analyzed by IBM SPSS Statistics 27.

RESULTS AND DISCUSSION

Chilling injury symptoms

Significant differences were observed in 'Big Top' fruits on different rootstocks for leatheriness and bleeding symptoms. However, in this experiment, 'Big Top' did not suffer severe CI symptoms after 28 days of cold storage plus 2 days at room temperature (Table 1), compared to a large collection of peach cultivars (Navarro et al., 2022). It has been found that nectarines have a better resilience to storage conditions than peaches (Manganaris et al., 2019). The highest and lowest browning index were reported for 'Adesoto 101' and 'PADAC 99-05', respectively, although they did not significantly differentiate from the rest of rootstocks. The highest bleeding index was reported for 'PADAC 04-03', followed by 'GF 677' and they were the rootstocks that increased the anthocyanins content the most in relation to harvest (data not shown). The highest mealiness index was found for 'PADAC 04-03' and

'ReplantPAC' although they did not statistically differentiate from the rest of rootstocks. Additionally, the highest leatheriness index was reported for 'PADAC 04-01' and 'PADAC 9902-01', although they did not differ significantly from 'GF 677'. The rest of rootstocks did not showed differences between them.

Table 1. Effect of rootstock on chilling injury (CI) symptoms after 28 days of cold storage at 5°C plus 2 days at room temperature.

Rootstock	Browning index	Mealiness index	Leatheriness index	Bleeding index
Adarcias	2.1±0.2 a	1.9±0.1 a	0.0±0.0 a	2.3±0.4 ab
Cadaman	2.5±0.2 ab	1.8±0.1 a	0.0±0.0 a	2.3±0.5 ab
GF677	2.8±0.2 ab	1.9±0.1 a	0.2±0.0 bc	3.1±0.2 bc
PADAC 99-05	2.1±0.1 a	1.9±0.1 a	0.1±0.1 ab	2.0±0.0 ab
PADAC 9902-01	2.4±0.2 ab	1.8±0.1 a	0.2±0.1 c	2.3±0.2 ab
ReplantPAC	2.3±0.5 ab	2.0±0.0 a	0.0±0.0 a	1.8±0.2 a
Adesoto 101	3.0±0.0 b	1.9±0.2 a	0.0±0.0 a	2.4±0.1 ab
PADAC 04-01	2.5±0.2 ab	1.4±0.0 a	0.2±0.0 c	1.9±0.3 a
PADAC 04-03	2.5±0.3 ab	2.0±0.4 a	0.0±0.0 a	3.6±0.6 c

Comparison means by Duncan's test ($P \leq 0.05$) are shown. Each value corresponded to the mean±the standard error (SE). For each column, different letters indicate significant differences.

Fruit quality

Rootstocks had a significant difference on fruit firmness and TA at harvest, as previously found in different peach-rootstock trials (Mestre et al., 2017; Reig et al., 2016). In addition, the cold storage treatment was highly significant for firmness and TA (Table 2) which decreased, in general, during storage and shelf-life (data not shown). The decline in TA was probably due to the consumption of organic acids in respiration (Liu et al., 2019). The decrease in firmness may be due to the fact that peach maturation continues after harvest.

Sugars and organic acids profile

Sugars and organic acids play an important role in peach taste perception. In descendent order, sucrose, glucose, sorbitol, fructose, myo-inositol and raffinose were quantified. Sucrose, glucose, fructose and sorbitol were the main individual sugars found as previously described for the 'Big Top' nectarine (Font i Forcada et al., 2019). In addition, malic, quinic, succinic + shikimic, citric, adipic, galacturonic + tartaric, propionic and formic acids were also quantified. Malic, quinic, succinic + shikimic and citric acid were the most abundant as previously reported (Font i Forcada et al., 2019).

The rootstock effect was statistically significant for total sugars (TS) but not the cold treatment (Table 2). The rootstock effect had a significant effect on glucose, fructose, raffinose and myo-inositol. Moreover, treatment effect was highly significant for glucose, sorbitol and raffinose. Glucose and sorbitol decreased during the cold treatment and raffinose increased (data not shown).

The rootstock effect was highly significant for total organic acids (TOA) and each individual organic acid, except for the formic acid (Table 2). The cold treatment did not affect TOA, but it was significant for each individual organic acid except for the propionic acid. Citric, malic and succinic + shikimic acids decreased during the cold treatment. In turn, galacturonic + tartaric, quinic, formic and adipic acid increased during the cold storage (data not shown).

Antioxidants

At harvest, the rootstock effect was significant for all the antioxidant compounds measured in fruits (Table 2). The cold storage treatment was also significant for RAC, TPC, vitamin C and anthocyanins. For most rootstocks, RAC, TPC and vitamin C decreased during the treatment (data not shown). RAC has been reported to increase first and then decrease during the cold storage (Liu et al., 2019). TPC decreased with fruit maturity (Cantín et al.,

2010) and it may be also due to phenolic compounds oxidation associated with browning (López et al., 2002). The anthocyanins increased for most rootstocks except in the case of ‘Cadaman’ and ‘PADAC 99-05’ (data not shown). Anthocyanins accumulation has been associated with bleeding (Manganaris et al., 2008). The health-promoting potential of fruits is due to vitamins, such as vitamin C, and secondary metabolites such as flavonoids (Weng and Yen, 2012). ‘Adesoto 101’ and ‘PADAC 04-01’ induced higher TPC and TFC, and ‘ReplantPAC’ and ‘PADAC 04-03’ induced the highest vitamin C concentrations at harvest and after the cold treatment (data not shown).

Table 2. Two-way ANOVA for linear model in fruit quality, antioxidants and enzymatic activity.

Trait	Rootstock	Treatment	Rootstock×Treatment
Firmness (N)	*	***	ns
SSC (mg SS g^{-1} DW)	ns	ns	ns
TA (mg MA g^{-1} DW)	*	***	*
Sucrose (mg g^{-1} DW)	ns	ns	ns
Glucose (mg g^{-1} DW)	***	***	ns
Fructose (mg g^{-1} DW)	***	ns	*
Sorbitol (mg g^{-1} DW)	ns	***	ns
Raffinose (mg g^{-1} DW)	***	***	ns
Myo-Inositol (mg g^{-1} DW)	***	ns	*
TS (mg g^{-1} DW)	*	ns	ns
Citric acid (mg g^{-1} DW)	***	*	ns
Malic acid (mg g^{-1} DW)	***	*	ns
Quinic acid (mg g^{-1} DW)	***	*	ns
Succinic + Shikimic acids (mg g^{-1} DW)	***	*	ns
Galacturonic + Tartaric acids (mg g^{-1} DW)	***	**	*
Formic acid (mg g^{-1} DW)	ns	***	ns
Propionic acid (mg g^{-1} DW)	***	ns	ns
Adipic acid (mg g^{-1} DW)	**	***	ns
TOA (mg g^{-1} DW)	***	ns	ns
RAC (mg TE g^{-1} DW)	***	**	ns
TPC (mg GAE g^{-1} DW)	***	**	ns
TFC (µg CAT g^{-1} DW)	***	ns	ns
AsA (µg AsA g^{-1} DW	***	***	***
AC (µg C3GE g^{-1} DW)	***	*	ns
PAL (U g^{-1} protein)	***	ns	ns
POX (U g^{-1} protein)	***	***	ns
PPO (U g^{-1} protein)	**	**	*

*P≤0.05, **P≤0.01, ***P≤0.001 and ns, not significant.

N, Newtons; DW, dry weight; SSC, soluble solids content; TA, titratable acidity; MA, malic acid; TS, total sugars; TOA, total organic acids; RAC, relative antioxidant capacity; TE, Trolox equivalent; TPC, total phenolics content; GAE, gallic acid equivalents; TFC, total flavonoids content; CAT, catechin; AsA, ascorbic acid; AC, anthocyanins content; C3GE, cyanidin-3-glucoside equivalents; PAL, phenylalanine ammonia-Lyase; POX, peroxidase; PPO, polyphenol oxidase; U, units of enzymatic activity.

Enzymatic activities

The rootstock effect was statistically significant for the activities of PAL, POX and PPO activities (Table 2). In addition, the cold storage treatment was significant for POX and PPO activities. In the case of ‘Adarcias’, ‘GF 677’, ‘PADAC 9902-01’, ‘ReplantPAC’ and ‘PADAC 04-01’ rootstocks, the POX activity seemed to decrease after 28 days at 5°C and increase after the two days of shelf-life (Figure 1A). On the other hand, PPO increased, in general, during the cold storage and the two days at room temperature, with the exception of ‘Adesoto 101’ (Figure 1B). The cold storage treatment was not significant for the PAL activity (Table 2). However, this activity seems to increase at 28 days as in the case of 'GF 677' and 'PADAC 04-01' (Figure 1C).

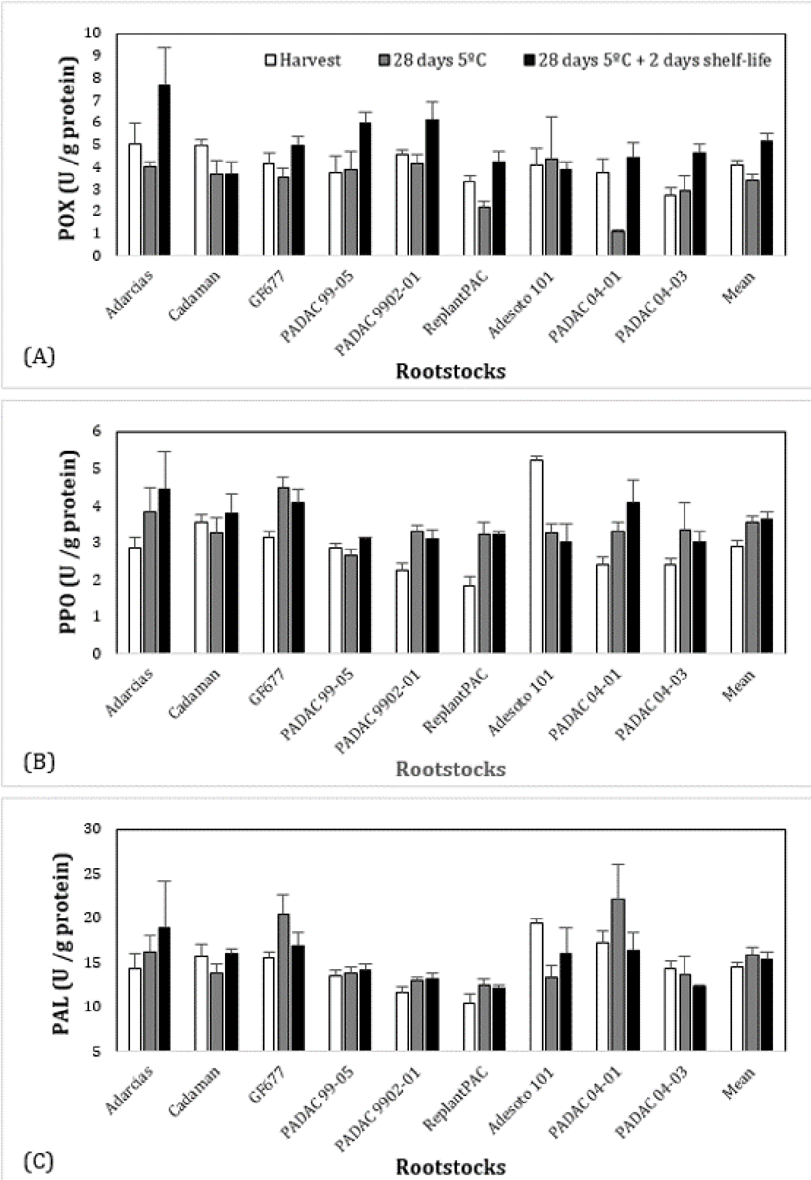

Figure 1. Enzymatic activities mean for each rootstock at harvest, after 28 days of cold storage at 5°C and after 28 days of cold storage plus 2 days at room temperature. (A) POX (U g^{-1} protein). (B) PPO (U g^{-1} protein). (C) PAL (U g^{-1} protein). Error bars refer to the standard error (SE) of the mean.

POX and PPO are main enzymes in ROS and phenolic compounds metabolism along with enzymatic browning and may be induced by chilling injury. The POX activity increase has been associated with color changes (López et al., 2002). PPO increases with browning due to phenolic compounds oxidation (Ogundiwin et al., 2007) and both, POX and PPO, also increase during maturation (López et al., 2002). PAL is an important enzyme involved in the phenylpropanoid pathway (Amri et al., 2021) and it has been associated with anthocyanins accumulation (Manganaris et al., 2008).

Correlation between fruit compounds and chilling injury symptoms

After 28+2d, soluble solids content was negatively correlated with browning (Table 3) which could be due to the role of some sugars as cryoprotectants and membrane stabilizators (Zhao et al., 2022).

Table 3. Pearson's correlation coefficients for chilling injury symptoms with enzymatic activity, antioxidants and fruit quality parameters.

Trait	Treatment (d)	Browning	Mealiness	Bleeding	Leatheriness
SSC (mg SS d^{-1} DW)	28+2[a]	-0.681*	ns	ns	ns
Sucrose (mg g^{-1} DW)	28[b]	ns	0.786*	ns	-0.764*
Sorbitol (mg g^{-1} DW)	28	ns	0.840**	ns	-0.667*
TS (mg g^{-1} DW)	28	ns	0.811**	ns	ns
Malic acid (mg g^{-1} DW)	Harvest[c]	ns	0.723*	ns	ns
Malic acid (mg g^{-1} DW)	28	ns	0.761*	ns	ns
Quinic acid (mg g^{-1} DW)	Harvest	0.854**	ns	ns	ns
Succinic + Shikimic (mg g^{-1} DW)	Harvest	0.749*	ns	ns	ns
Succinic + Shikimic (mg g^{-1} DW)	28	ns	0.727*	ns	ns
Succinic + Shikimic (mg g^{-1} DW)	28+2	0.716*	ns	ns	ns
TOA (mg g^{-1} DW)	Harvest	0.720*	ns	ns	ns
TOA (mg g^{-1} DW)	28	ns	0.744*	ns	ns
TPC (mg GAE g^{-1} DW)	(28+2)-28[d]	0.779*	ns	ns	ns
AC (µg C3GE g^{-1} DW)	Harvest	ns	ns	0.830**	ns
AC (µg C3GE g^{-1} DW)	28	ns	ns	0.758*	ns
AC (µg C3GE g^{-1} DW)	28+2	ns	ns	0.721*	ns
PAL (U g^{-1} protein)	Harvest	0.669*	ns	ns	ns
PPO (U g^{-1} protein)	Harvest	0.674*	ns	ns	ns
POX (U g^{-1} protein)	(28+2)-28	-0.703*	ns	ns	ns

[a]28 days at 5°C plus 2 days at room temperature. [b]28 days at 5°C without shel-life. [c]No cold treatment. [d]The difference between 28 days at 5°C plus 2 days at room temperature and 28 days at 5°C without shelf-life.* indicates P≤0.05, ** P≤0.01, and ns not significant. Only Trait x Treatment combinations showing significant correlations with CI traits are in the table. DW, dry weight; SSC, soluble solids content; TS, total sugars; TOA, total organic acids; TPC, total phenolics content; GAE, gallic acid equivalents; AC, anthocyanins content; C3GE, cyanidin-3-glucoside equivalents; PAL, phenylalanine ammonia-lyase; PPO, polyphenol oxidase; POX, peroxidase; and U, units of enzymatic activity.

After 28d of cold storage without shelf-life, TS, sorbitol and sucrose were positively correlated with mealiness. In the other hand, sorbitol and sucrose were negatively correlated with leatheriness. Sucrose helps in antioxidant defense and membrane stabilization and sorbitol acts as osmotic protectant (Zhao et al., 2022). At harvest, TOA, succinic + shikimic and quinic acids were positively correlated with browning. Moreover, malic acid was positively correlated with mealiness.

The decrease of TPC (TPC after 28 days at 5°C plus 2 of shelf-life minus TPC after 28 days without shelf-life) was positively correlated with browning. The decrease of phenolic compounds was higher in 'Adesoto 101', the rootstock that induced higher browning symptoms. Browning has previously been associated with total phenolics compounds and it is mainly attributed to their oxidation (Liu et al., 2019).

The anthocyanins content at harvest, after the cold treatment and after shelf-life was positively correlated with bleeding. It means that fruits with higher pigmentation at harvest have maintained or even increased the levels of anthocyanins. It could be considered as bleeding when anthocyanins significantly increase, although they could also increase with fruit maturation (Manganaris et al., 2008).

At harvest, PAL and PPO activities were positively correlated with browning (r=0.792*). PAL is involved in the biosynthesis of the polyphenolic compounds and PPO participates in their oxidation. The rootstocks with higher PAL activity at harvest were 'Adesoto 101' and 'PADAC 04-01' that were also the rootstocks that induced higher TPC. The increase of the POX activity during fruit shelf-life was negatively correlated with browning. A higher activity of the peroxidase could indicate it has not still react, thus phenols have not yet been oxidized.

Moreover, mealiness was negatively correlated with leatheriness (r=-0.723*) because mealiness is characterized by a woolly texture while leatheriness is described as a hard texture (Lurie and Crisosto, 2005).

CONCLUSIONS

A significant influence of the evaluated *Prunus* rootstocks was found concerning basic fruit quality traits, antioxidants, enzymatic activity and CI symptoms in 'Big Top' fruits, both at harvest and/or after cold storage of fruits at 5°C. At harvest and after cold storage, 'Adesoto 101' and 'PADAC 04-01' induced the highest relative antioxidant capacity and 'ReplantPAC' and 'PADAC 04-03' induced the highest vitamin C concentrations, all of these rootstocks having a plum genetic background. Therefore, these rootstocks could increase ROS scavenging and health-promoting potential of the 'Big Top' nectarine.

Although low susceptibility to CI symptoms was found in the 'Big Top' nectarine (after 28 days of cold storage plus 2 days at room temperature), 'Adarcias', 'PADAC 04-01', 'PADAC 99-05' and 'ReplantPAC' rootstocks generally had lower CI symptoms. Moreover, organic acids, anthocyanins and the enzymatic activities of PAL and PPO have demonstrated to be important parameters to take into account in the prevention of chilling disorders. These results improve the knowledge of nectarines performance grafted on different rootstocks and will be useful to the choice of rootstocks, in particular to promote a lower chilling injury susceptibility.

ACKNOWLEDGMENTS

This work was funded by the Spanish Ministry of Science Innovation and Universities (MICINN) grant RTI2018-094176-R-C33, cofounded by FEDER, and the Regional Gov. of Aragon (A44, T07_17R, A22_20R).

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