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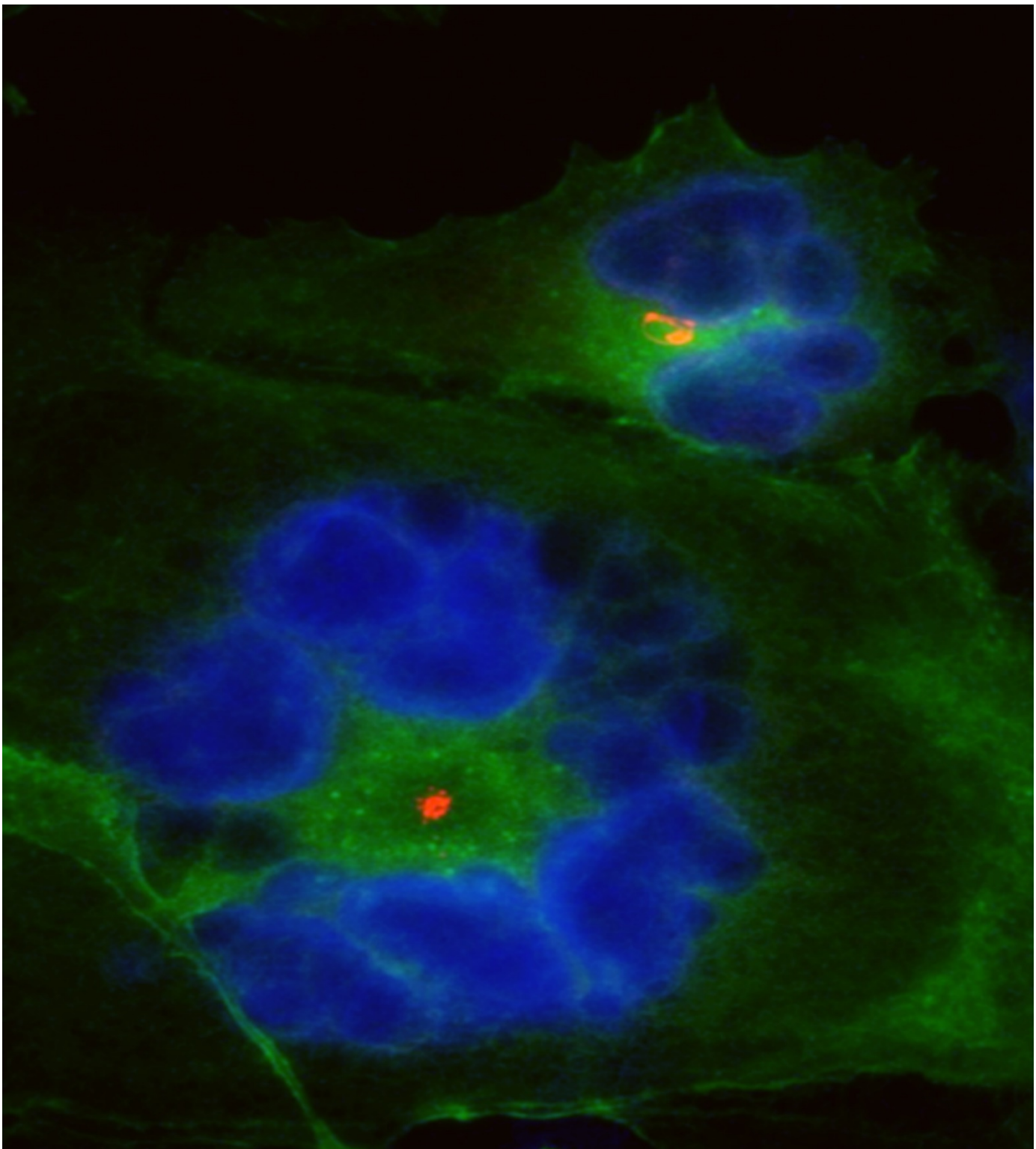

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Cover page: The Synthetic Lethal Rosette

Aberrant mitotic phenotype found in BRCA1-deficient cells treated with the PLK1 inhibitor Volasertib. Cells become giant and multinucleated and acquire a flower shape, with nuclei arranging in a circular disposition around a cluster of centrosomes. Blue (DAPI: nuclei), Green (FITC-phalloidin: actin cytoskeleton), Red (γ-Tubulin: centrosomes).

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PL-P40
ZMS5H, A NOVEL ENZYME INVOLVED IN SALICYLIC ACID HYDROXYLATION

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Salicylic acid (SA) has been described as an important signaling molecule in plants, regulating growth, development, senescence, and responses to biotic and abiotic stresses. Levels of salicylic acid are regulated not only by activation of its biosynthetic pathway, but also through its modification by metabolic modifications, such as glycosylation, methylation, amino acid conjugation, and hydroxylation. Hydroxylated SA is the major degradation product of SA. Recently, the *Arabidopsis thaliana* enzyme catalyzing SA to 2,3-dihydroxybenzoic acid (2,3-DHBA; *At*S3H) has been identified, and SA was found to accumulate in *s3h* mutants. In this study, we report the discovery and functional characterization of a novel maize salicylic acid 5-hydroxylase (*Zm*S5H), a 2-oxoglutarate dependent dioxygenase that catalyzes the formation of 2,5-DHBA by hydroxylating SA at the C5 position of its phenyl ring. Once identified, we carried out *in vitro* activity assays in order to kinetically characterize this enzyme. His-tagged *Zm*S5H was heterologously expressed in *Escherichia coli* and then purified. The reaction product 2,5-DHBA was identified by HPLC by comparison with authentic standards. Interestingly, according to sequence similarity analysis, *Zm*S5H and *At*S3H are closely evolutionarily related, though we could not identify 2,3-DHBA as a product of the studied reaction. Kinetic parameters of the recombinant *Zm*S5H were also determined by HPLC. In addition, its activity *in planta* was demonstrated, as transgenic *Arabidopsis* plants expressing *Zm*S5H were more susceptible to *Pseudomonas syringae* pv. *tomato DC3000* pathogen infection than WT plants, suggesting that these plants would have decreased SA levels due to higher hydroxylation of the hormone. In order to further confirm this, we investigated the expression level of three different genes modulated by SA in *s3h*, wild-type and transgenic Arabidopsis plants expressing *Zm*S5H that were treated with this hormone compared to plants treated with a mock solution. *PR1*, *EDS-1*, and *SAG13* showed a decreased expression level in plants overexpressing *Zm*S5H compared to *s3h* mutants and wild-type plants, suggesting that in fact, *Zm*S5H hydroxylates SA *in planta*. We are now analyzing the possible crosstalk between SA hydroxylation and flavonoids synthesis, a model proposed in our laboratory, based on previous results. So far, transgenic plants expressing two different maize flavone synthases recently characterized, (*ZmFNSI* and *ZmFNSII*), which accumulate flavones, in a mutant *s3h* background, show increased susceptibility towards infection with *P. syringae* compared to wild-type and mutant plants, suggesting that flavones regulate SA levels *in vivo*.

PL-P41
EFFECT OF FOLIAR APPLICATION OF PHOSPHITES IN "HAYWARD" KIWIFRUIT IN STORAGE AND SHELF LIFE: ANALYSIS OF PECTIN COMPOSITION

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The kiwifruit (*Actinidia chinensis* var *chinensis* cv. Hayward), a climacteric fruit, can be harvested at physiological maturity and maintain its quality for up to six months at cold storage. The length of the storage period depends, among others, on cell wall composition and structure, which impacts on texture and softening. Indeed, solubilization and degradation of pectins occur during fruit softening, leading to the disintegration of the cell wall. Pectin is a complex heterogeneous polymer that can have different interactions within the cell wall as free pectin, bound to starch, attached by calcium bridges and bound to cellulose via hydrogen bonds. The phosphites activate the synthesis of compounds that reinforce cell walls, like pectin and lignin. The aim of this work was to study the effect of foliar application of phosphite on pectin composition in cold storage and shelf life. Plants were foliar sprayed (six weekly applications) 100 days after blooming, with 0.3% potassium phosphite (KPhi; 30% P_2O_5, 20% K_2O) or water (Control). Fruits were harvested at physiological maturity and stored for 5 and 6 months (5M and 6M) at 0°C and 90–95 % RH. Kiwifruit was analyzed at the end of each storage period (ES) and its shelf life (SL, 7 days at 20°C). Samples of outer pericarp tissue were frozen and ground using liquid N_2. A chemical solvent method was used to successively extract cell walls and determine the composition of pectin. The cell wall material (CWM) was obtained by the inactivation of the enzymes with a mixture of phenol/acetic acid/water (PAW) and water-soluble pectin fraction (W-SP) was recovered. To remove the kiwifruit starch and extract their bound pectin (S-SP), a solution of dimethyl sulfoxide was used. The Na_2CO_3 was added to obtain the pectin attached by tightly bonds and calcium bridges (C-SP). Each extract was dialyzed 5 days, lyophilized, and weighed. The results showed that KPhi treatment increased the yields of total pectins respect to the Control and also resulted lower at 5M respect to 6M of storage. At shelf life, pectin yield decreased in all treatments compared to the end of storage. The yield of W-SP fractions was lower in SL than in ES and resulted highest in KPhi treatment at 6M. The yield S-SP fraction was lowest in KPhi and SL. This could be due to the enzymatic degradation of starch. The proportion of C-SP yield also was lower in KPhi but was higher in SL. The yield of CWM decreased from the 5M to 6M and increase at SF with the application of KPhi. These results suggest that KPhi treatment promotes the pectin biosynthesis and their release in shelf life. After 5–6 months of cold storage, "Hayward" kiwifruit enters in its last ripening/over-ripening stage related to senescence, led by the cell wall disintegration. In conclusion, KPhi treatment is suggested to be used in order to maintain the firmness "Hayward" kiwifruit, at least until 5 months in cold storage prior shelf life and its consumption.