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Effects of Hydrogen Peroxide on Growth, Development and Quality of Fruits: A Review

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ABSTRACT

There is increasing concern about the effects of hydrogen peroxide (H_2O_2) on plant growth and development as well as fruit quality, worldwide. Hydrogen peroxide is produced predominantly in plant cells during photosynthesis and photorespiration and also in respiration processes. It is most stable of so-called Reactive Oxygen Species (ROS) and therefore, plays crucial role as signaling molecule in various physiological process. Increment of intra- and intercellular levels of H_2O_2 give effect on growth, development and quality of fruits with the optimum concentration of H_2O_2 application. In this study the effects of H_2O_2 on growth, development and quality of importance fruit production were discussed. The past research was also discussed about the effects of H_2O_2 on germination of seedling until maturation, flowering and fruiting stage and fruit quality during pre-harvest and postharvest storage behavior.

Key words: Hydrogen peroxide, growth, development, fruit quality, reactive oxygen species, fuji apple

INTRODUCTION

Hydrogen peroxide (H_2O_2) is a strong oxidant that long has been used in industrial application and water treatment processes. When catalysed in water, H_2O_2 may generate a wide variety of free radicals and other reactive species that are capable of transforming or decomposing organic chemicals (Petri *et al*., 2003). It is an environment friendly compound where, it is predominantly produced in plant cell during photosynthesis and photorespiration and to a lesser extent, in respiration (Slesak *et al*., 2007).

During photosynthesis, the plants used carbon dioxide (CO_2) and water (H_2O) and produced carbohydrate ($C_6H_{12}O_6$) and released oxygen (O_2) as byproduct, while, during respiration, the carbohydrate are converted into energy where, the energy is used in the process of building new tissues. Photosynthesis required light while, respiration can occur anytime, either in the dark or light. The process of both photosynthesis and respiration are summarized in Fig. 1.

The presence of O_2 in the earth's atmosphere is believed originates from photosynthesis activity (Slesak *et al*., 2007). Oxygen in the ground state is a molecule with two unpaired electrons, each located in a different π* anti-bonding orbital and divalent reduction of O_2 is not a simple process and requires the generation of univalent intermediates. The addition of single electron requires an energy input and reduces O_2 to the superoxide anion radical (Eq. 1). Since the extra electron is in an unpaired state in the outer orbital, the superoxide is a free radical. It is relatively unstable, being either converted back to O_2 or in a reaction catalysed by the enzyme superoxide dismutase (SOD) (Eq. 2):

$$O_2 + le^{*-} \rightarrow O_2^- \quad (1)$$

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \quad (2)$$

In contrast to the superoxide, H_2O_2 belongs to non-radical Reactive Oxygen Species (ROS) and is a molecule that carries

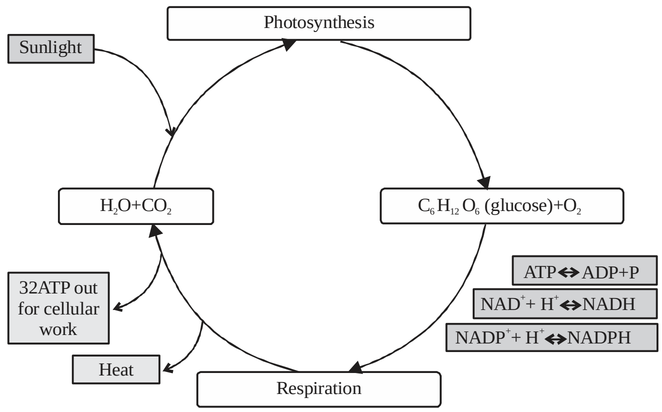

Fig. 1: Process of photosynthesis and respiration

no net charge (Halliwell, 2006). Because of this and the larger half-life of H_2O_2 than that of the superoxide anion radical, hydrogen peroxide is more likely to be a long-distance signaling molecule than superoxide (Vranova *et al*., 2002). This is supported by Neill *et al*. (2002) where, H_2O_2 is generated via superoxide, presumably in a non-controlled manner, during electron transport processes such as photosynthesis and mitochondrial respiration.

Meanwhile, Slesak *et al*. (2007) suggested that H_2O_2 plays a crucial role as a signalling molecule in various physiological processes, including photosynthesis, respiration, translocation and transpiration as hydrogen peroxide is most stable Reactive Oxygen Species (ROS). Thus, these processes will lead to increment of crop yield and productivity.

Recently, the usage of H_2O_2 as plant growth promoting chemicals is widely being used by farmer in small-scale and large-scale farming. It has been regarded as a signalling molecule and regulator of the expression of some genes in cells (Quan *et al*., 2008). It also reported that H_2O_2 have regulatory effects on growth, development and quality of fruit.

PLANT GROWTH AND DEVELOPMENT

Plant growth and development will be affected by severe environmental conditions, including various biotic and abiotic stresses. During evolution, plants have evolved complex regulatory mechanisms to adapt to various environmental stressors. One of the consequences of stress is an increase in the cellular concentration of ROS, which are subsequently converted to H_2O_2.

Even under normal conditions, higher plants produce ROS during metabolic processes. Excess concentrations of ROS result in oxidative damage to or the apoptotic death of cells. Development of an antioxidant defense system in plants protects them against oxidative stress damage. These ROS and more particularly, H_2O_2 play versatile roles in normal plant physiological processes and in resistance to stresses (Quan *et al*., 2008).

The development of a mature plant from a single fertilized egg requires precise and highly ordered succession of events. The fertilized egg cell or zygote divides, grows and differentiates into increasingly complex tissues and organs. In the end, these events give rise to the complex organization of a mature plant that flowers, bears fruit, senesces and eventually dies. These events, along with their underlying genetic programs and biochemistry and the many factors that either impose or modulate an unfailing and orderly progression through the life cycle, constitute development (Hopkins and Hunter, 2004).

The changes that an organism goes through its life cycle: From germination of the seed through growth, maturation, flowering, seed formation and senescence, applies equally to cells, tissues and organs, each experiencing similar patterns of change. The changes can be seen on form of the organism or organ, such as the transition from the vegetative to flowering condition of from leaf primordium to fully expanded leaf (Hopkins and Hunter, 2004). It is important for fruit growers to have information on the differences in fruit quality parameters over time. It has been reported that Plan Growth Regulators (PGRs) enhance the rapid changes in physiological and biochemical characters and improve crop productivity (Khandaker *et al*., 2012a).

Plant growth, development and senescence in many plants are accelerated by plant growth regulators, growth promoting chemicals and ethylene (Khandaker *et al*., 2012b). It is also occur at the subcellular and biochemical levels, such as when chloroplasts appear in leaf cells brought into the light and the enzymes of photosynthesis are activated.

Changes in endogenous cytokinins have an important role in flower induction in some plants (Moneruzzaman *et al*., 2011b). The changes in size and mass, whether fresh weight, dry weight, length and perhaps width would be suitable measure of growth. For the commercial grower and for a person, who wants to grow the fruits in home garden, it is very important to have information on the differences in fruit growth, development as well as nutritional quality of fruits (Moneruzzaman *et al*., 2011a).

Effect of H_2O_2 on the germination and early seedling of fruit trees: Slow growth of seedlings under natural conditions is a horticultural problem where, this problem can be solved by applying hydrogen peroxide (H_2O_2) as a treatment in order to increase the germination percentage of seeds as well as the growth of seedlings in a concentration-dependent manner (Barba-Espin *et al*., 2010). Hydrogen peroxide, an extracellular ROS is significantly produced during seed germination and early seedling development and also during seed aging (Kranner *et al*., 2010).

Seed began to absorb and swell immediately after placement in water and gained approximately 40% in weight within 40 h with little additional uptake in the next 60 h (Alexander, 1966). This should be the best time to apply H_2O_2 by mixing it with the water that used for irrigation. The H_2O_2-pretreatment produced an increase in ascorbate peroxidase (APX), peroxidase (POX) and ascorbate oxidase (AAO) (Barba-Espin *et al*., 2010). The increases in these ascorbate-oxidizing enzymes correlated with the increase in the growth of the pea seedlings as well as with the decrease in the redox state of ascorbate. On the other hand, H_2O_2 relatively lightened abscisic acid (ABA) inhibitions on the radicle elongations and fresh weights while, it had no effect on ABA suppression on the emergence percentage and elongation of the coleoptile (Cavusoglu and Kabar, 2010).

The application of H_2O_2 during early stage of plants also gave positive effect to plants by enormously removed the germination-delaying and inhibiting effects of temperature increases (Cavusoglu and Kabar, 2010). Hydrogen peroxide-pretreatment became very successful in the overcoming of the germination-delaying and preventing effects of the increases both salt and temperatures levels. It markedly alleviated the inhibitions of salt on seedling growth at all temperatures as well.

The proteomic analysis showed that H_2O_2 induced proteins related to plant signalling and development, cell elongation and division and cell cycle control (Barba-Espin *et al*., 2010). Therefore, there is an interaction among the redox state and plant hormones, orchestrated by H_2O_2, in the induction of proteins related to plant signalling and development during the early growth of seedling.

Effect of H_2O_2 on leaf of fruit trees: Most leaves appear simple at first sight. As leaves are initiates, meristem cell divide and replace the cells that have just been committed to initiating a leaf primordium. The regular pattern of leaf initiation allows one to predict, where the next leaf will appear (Micol and Hake, 2003). Cell wall hydration in leaves allows cell wall extension through structural alteration. While, relaxing the cell wall stretches the plasma membrane, which promotes opening of Ca^{2+} channels. This resulting increases in cytoplasmic calcium affects growth by inhibiting P-ATPases and also activates NADPH-oxidase, which promotes secretion of superoxide into the cell wall, which further converted into H_2O_2 (Kalve *et al*., 2014).

Hydrogen peroxide was detected cytochemically by its reaction with cerium chloride, which produces electron-dense deposits of cerium perhydroxides. Hydrogen peroxide was present in the plasma lemma of the phloem cells of recovered apricot plant leaves, but not in the asymptomatic or symptomatic material. Furthermore, by labelling apricot leaf tissues with diaminobenzidine (DAB), no differences were found in the localization of peroxidases (Musetti *et al*., 2005). Therefore, H_2O_2 and related metabolites and enzymes appear to be involved in lessening both pathogen virulence and disease symptom expression in European Stone Fruits Yellows (ESFY)-infected apricot plants.

Effect of H_2O_2 on flowering and bud formation: Flower initiation marks the transition from vegetative to reproductive growth in seed plants (Zhou *et al*., 2012). It is thus a crucial event in the life of these plants, because it has peculiar relation of vegetative and reproductive development in seed plants, which is in turn an outcome of the morphological nature of the flower. Flowers are modified shoots, which are produced by modified shoot meristems, flower primordia. However, once a meristem has been determined as a flower primordium, it is perhaps the very earliest stages of reverting to vegetative growth, where vegetative growth and reproductive development in seed plants are in a certain sense mutually exclusive. As far as a particular meristem is concerned, flower initiation means the end of its life (Lang and Nitsch, 1965).

They were strongly competing with each other during panicle development in flowering and bud formation of fruit, while, hydrogen peroxide (H_2O_2) and nitric oxide (NO) play important roles in the competition to stimulate reproductive growth by inhibiting the growth of rudimentary leaves as well as by promoting the expression of the flower related gene, *LcLFY* in litchi (*Litchi chinensis* Sonn.) (Zhou *et al*., 2012).

The chilling-induced flowering increased H_2O_2 and NO contents in the mixed buds of litchi (*Litchi chinensis* Sonn.) (Zhou *et al*., 2012). Bud usually may remain for some time in a dormant condition, once it is formed. However, initial bud drop is a serious problem in fruit production. The bud drop of wax apple fruit (*Syzygium samarangense*) can be reduced by applying the trees with 20 mM H_2O_2 (Khandaker *et al*., 2012a).

Effect of H_2O_2 on fruit growth and development: Fruit is result from maturation of one or more flowers and gynoecium of the flowers form all part of the fruit (Mauseth, 2003). The fruit growth can be enhanced by spraying the crop with H_2O_2 treatment. Spraying wax apple trees once a week with 5 mM H_2O_2 to get better fruit growth. It showed significantly increased with the photosynthetic rates, stomatal conductance, transpiration, chlorophyll and dry matter content of the leaves and total soluble solids and total sugar content of the fruits of wax apple (*Syzygium samarangense*) (Khandaker *et al*., 2012a).

Hydrogen peroxide may enhance cellular development during initial cell division at phase I or modulate cell expansion at phase II by its cell wall loosening effect (Geros *et al*., 2012). The treated wax apple fruit with 5 mM H_2O_2 showed larger fruit size, increased fruit set, fruit number, fruit biomass and yield compared to the control (Khandaker *et al*., 2012a) while, the presence of H_2O_2 in the fruit could promote the ripening process (Geros *et al*., 2012).

QUALITY OF FRUIT

The impact of food quality on human health has been increasing in public interest. In the olden days, the agricultural industry was focused on maximizing the production of the quantity of fruits for commercial markets. However, now a days, the modern consumers are now interested in optimizing the nutritional composition of foods in order to promote health (Wang, 2010). Therefore, much attention has now been placed on the agricultural practices, which will enhance the nutritional content of horticultural crops being produced today.

Fruits have been shown to contain high levels of antioxidant compounds such as carotenoids, vitamins, phenols, flavonoids, dietary glutathione and endogenous metabolites. These antioxidants can act as free radical scavengers, peroxide decomposers, singlet and triplet oxygen quenchers, enzyme inhibitors and synergists (Wang, 2010). The various antioxidant components found in fruits, such as banana, may provide protection against cancer and heart disease (Agoreyo, 2012; Wang, 2010) in addition to a number of other health benefits.

Effect of H_2O_2 on fruit quality during pre-harvest: The antioxidant content and antioxidant activity will be affected by the pre-harvest conditions such as climate, temperature, light intensity, soil type, compost, mulching, fertilization, increasing carbon dioxide concentration in the atmosphere and application of naturally occurring compounds. With regard of these factors, the K(+), anthocyanin and carotene contents, flavonoid, phenol and soluble protein content, Sucrose Phosphate Synthase (SPS), Phenylalanine Ammonia Lyase (PAL) and antioxidant activities were increased in the treated wax apple fruits with concentration 20 mM of H_2O_2 (Khandaker *et al*., 2012a). When comparing between peel colour (hue) and Total Soluble Solids (TSS), between net photosynthesis and SPS activity and between phenol and flavonoid content with antioxidant activity, there was a positive correlation in H_2O_2-treated fruits (Khandaker *et al*., 2012a).

Another important factor determining fruit quality is sweetness. Sweetness and sugar composition can be measured by using standard hand-held refractometer, hydrometer, electronic tongue and High Pressure Liquid Chromatography (HPLC) equipped with different detectors (Magwaza and Opara, 2015). Ozaki *et al*. (2009) recently reported that the application of H_2O_2 enhanced sweetening in melon fruits. The improvement in fruit quality will increase the value of the fruit and thus, will expand its industry.

The harvesting time or date is very importance in determining the susceptibility of any disease to fruit. Early harvest fruits had the highest injury incidence and severity as well as H_2O_2 and malondialdehyde (MDA) contents, while late harvest had the lowest for 'Fuji" apple (Lu *et al*., 2014). Meanwhile, golden delicious apples (*Malus domestica*) is best to be picked seven days before the commercial harvest time as the H_2O_2 level is significantly increased with the increasing of superoxide dismutase activity (SOD) (Torres *et al*., 2003).

The harvested fruits also need optimum temperature for storage. All 'Fuji" apple fruits showed a rapid increase in injury incidence and severity after warming, especially at the first 4 days, when H_2O_2 and MDA levels and polyphenol oxidase (PPO) activities also increased. They were relatively resistant to injury and that injury symptom development was highly dependent on the accumulations of H_2O_2 and MDA while PPO activity remained constant during storage at 0ºC (Lu *et al*., 2014). Therefore, "Fuji" apple is best to be stored at 0^oC.

Effect of H_2O_2 on postharvest of fruit quality: The quality of fruit can be improved only at production level, but after harvesting, the fruit can only be maintained its quality. Factors such as genetic, geographic location, environmental conditions and pre-harvest cultural practices including canopy management, nutrition and irrigation management have been reported to influence post-harvest disease development and quality attributes of mango (Rehman *et al*., 2015).

For maintaining the harvested fruits, there is need a proper handling in order to keep their quality, general appearance, long lifespan for storage time and maintained some nutritional value as well as reduced decay development during storage condition. Hydrogen peroxide can be used as a sanitizing treatment on fresh-cut pineapple stored at 5°C as there is no significant differences in microbial counts, physiochemical values and sensory attributes were observed between samples left untreated or treated with 1 or 3% of H_2O_2 (Aida *et al*., 2011). Meanwhile, fresh-cut pineapple treated with 3% H_2O_2 had the highest lightness value and maintained flesh firmness better than 1% H_2O_2 (Aida *et al*., 2011).

CONCLUSION

From the previous discussion, it can be concluded that hydrogen peroxide has a great effect on the fruit growth and development as well as quality of fruits. Hydrogen peroxide help in overcoming the germination-delaying by removing the blockage of abscisic acid (ABA) and involve in lessening both pathogen virulence and disease symptom expression in some plants. Hydrogen peroxide also promote reproductive growth by inhibiting the growth of rudimentary leaves as well as by promoting the expression of the flower related gene, *LcLFY* and reducing the bud drop. With the optimum concentration of H_2O_2, the K (+), anthocyanin and carotene contents, flavonoid, phenol and soluble protein content, Sucrose Phosphate Synthase (SPS), Phenylalanine Ammonia Lyase (PAL) and antioxidant activities in the treated fruits are increasing. Thus, the fruits are marketable and meet the market demand. Last but not least, H_2O_2 can be used a sanitizer for a fresh cut fruits while, maintaining the flesh firmness. Therefore, the application of H_2O_2 is a new tool that can be used in horticulture industry.

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