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Review

An overview of tomato fruit-ripening mutants and their use in increasing shelf life of tomato fruits

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The potential of tomato ripening mutants; rin, nor, alc, nr, gr, etc to prolong the shelf life of tomato cannot be overemphasized. Ripening mutants have gained a considerable attention in the last few years to extend shelf life. They have been characterized and examined for their potential utilization in lengthening shelf life of tomatoes. Mutant genes are therefore available to extend the shelf-life of tomato via breeding. These single recessive gene mutants mostly modify ethylene's downstream effects on specific biochemical processes related to fruit ripening. The fruits of these mutants are characterized by an absence of a ripening-associated ethylene burst and failure to ripen in the presence of exogenous ethylene. They are useful in research and breeding of cultivated tomatoes for postharvest quality. The effect is more pronounced in homozygotes where fruits do not develop normal colour. However, in the heterozygous form, several ripening characteristics exhibit levels that are intermediate between those of the wild and mutant parents and are able to produce fruits that have high shelf life than normal cultivars. Their discoveries have generated insights into ripening control and created new understanding of the primary ripening control mechanisms. Nonetheless, some scientists have argued that these mutant genes produce undesirable pleiotropic effects on other components of fruit quality. This is true in the homozygotes state, however in the heterozygote form, they develop acceptable colour for marketing, ripe naturally and exhibit extended shelf life too. Hence this offers an opportunity to exploit these genes to regulate tomato supply for longer period.

Key words: Ethylene, fruit ripening, ripening mutants, shelf life, tomato.

INTRODUCTION

Tomato (*Solanum lycopersicum* L. 2n=24*)* is one of the most widely cultivated and consumed vegetable crops in the world (Grandillo et al*.,* 1999) and is the second important vegetable crop after potato in the world (Saeed et al*.,* 2014). Tomatoes and tomato-based foods offer a wide variety of nutrients and lots of health-related benefits and can be eaten raw as fruits, salads or use as ingredient in the preparation of stews, soups, drinks and many other dishes (Alam et al., 2007). In Africa and especially Ghana where it is cultivated and consumed

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daily in every household, it constitutes a very important part of people's food (Wolff, 1999; Osei et al*.,* 2010). Wolff (1999) points out that because tomato is consumed daily in every household, consequently, it requires more money to purchase than any other vegetable. Production of tomato is thus an important source of income for most rural and peri-urban producers in most developing countries who are the main producers of this crop. Regardless of all the many benefits from the crop, many challenges beset its production. The challenges faced by tomato farmers and other stakeholders are attributed to a number of constraints in the tomato production, postharvest handling and marketing chain or a blend of them. A major dilemma in contemporary tomato production is postharvest handling. Tomato experiences great postharvest losses due to its natural perishability, precarious transportation, storage conditions and improper packaging. Kitinoja and Kader (2015) reported that the postharvest losses of fruits and vegetables in the developing countries account for almost 50% of the produce. Arah et al. (2015) further emphasized that postharvest losses in tomatoes can be as high as 25 to 42% globally. A fundamental limitation in fruit marketing is the premature ripening and softening during transportation. This predisposes the crop to rapid post-harvest softening and poor shelf life leading to great losses. Arah et al. (2015) indicated that these losses 'bring low returns to growers, processors and traders'. The authors again emphasized that this will result in loss of foreign exchange earnings in Ghana. Shelf-life is therefore an important quality trait that influences fresh tomato marketability, transportation and domestic use. Firm tomato fruits are also demanded by growers, shippers and processors to enable the fruit to withstand the rigors of shipping and reach the consumer in acceptable condition.

Several postharvest methods and treatments have shown some decline in fruit decay and weight loss in tomato. Such treatments include the use of gibberellic acid, calcium chloride, potassium and salicylic acid with chlorine (Mujtaba et al*.,* 2014). Other methods include packing in perforated polyethylene bag and kept at ambient condition. These methods are however, quite laborious and impracticable in farmers' fields. Additionally, advanced technique RNA interference can be used to down regulates the genes involved in ethylene biosynthesis and cell-wall degrading enzymes to extend the shelf life in tomato (Yogendra and Gowda, 2013; Carrari et al*.,* 2007). However, this involves genetic modification which is not supported by some countries due to lack of environmental safety and social acceptance of genetically modified crops. A high yielding tomato cultivar with high quality fruit and a long shelf life is one of the main goals in any fresh market tomato breeding program. Fresh-market tomatoes often have poor consumer quality, which is due in part to the marketing procedures used. Fruits are picked green and ripening is initiated by ethylene treatment for fresh market. This practice can produce satisfactory or acceptable fruit when tomatoes are picked mature-green, but often immature green fruits are also harvested. The immature green fruits will develop red pigmentation without acquiring the flavor, texture, and quality of a vine-ripe tomato (Kader et al*.,* 1977). This commercial practice is developed to facilitate tomato harvest, prolong fruit shelf life and minimize physical damage to the fruit during handling. The regulatory events controlling fruit ripening have greatly benefitted from the availability of natural ripening mutants. Use of the ripening mutant genes in the heterozygous condition offers the possibility of picking fruit at early stages of red pigment development, thereby ensuring harvest of mature fruit, and yet having adequate shelf life (Kopeliovitch and Rabinowitch, 1979; McGlasson et al*.,* 1983; Tigchelaar et al*.,* 1978). The present review discusses commercially used ripening mutants as potential germplasm for prolonging shelf-life of cultivated tomato and emphasizes the characteristic expression and effects of *rin*, *nor* and *alc*. It also highlights the prospects and problems associated with using the mutants.

TOMATO FRUIT DEVELOPMENT

Tomato fruit's development is initiated by fertilization (Picken, 1984; Gillaspy et al*.,* 1993). Fruits can be formed when the yellow flowers on the tomato plants are fertilized. As soon as they are fertilized, the flowers develop into small green globes that become visible at the base of the blossoms and ultimately become mature fruits (tomatoes). Tomato plants possess both the male and female reproductive parts often referred to as monoecious. The male reproductive part is structured in such a way that inside each flower, pollen from the male stamen is able to pollinate the female pistil thereby ensuring fertilization. Though this does not happen in every single flower on each plant, the process can be aided by agents such as insects (eg. Bees), wind and Man. The insects find themselves inside the flowers for nectar and move the pollen from one place to the other. Man and wind also ensure pollination by shaking the plants in an effort that causes the pollen grains to disperse.

A single tomato branch can have many or few yellow flowers with the prospective flower first appearing on the end of the branch as a small tightly closed bud contained by outer leaves designated as sepals, also collectively known as the calyx. Right inside the calyx, the yellow petals, or the corolla, of the tomato flower develop around its reproductive organs composed of the (pistal) that houses the bright green carpels where fruit formation takes place following fertilization. Once fertilized, the yellow petals wither and fall off to make way for the developing fruit. That is, the flower begins to age, a

process called senescence, shedding its petals and female reproductive organs, revealing the tiny green tomato still attached to the sepals. At this stage, unfertilized flowers are however, excluded by the plant and separated just below the sepals. Even though flowers in a cluster do not develop and mature at the same rate, it is common to see unopened buds, mature blossoms and developing tomatoes on a single branch at the same time. The ovary wall is transformed into the pericarp as soon as fertilization is successful. The pericarp can be divided into three different structures: exocarp, mesocarp, and endocarp. The external exocarp is made up of a cuticle layer and the skin. The cuticle layer thickens as the fruit ages. The skin however, contains an epidermal cell layer and a collenchymatous tissue (three to four layers), in which starch accumulates and few plastids are retained (Esau, 1953; Varga and Bruinsma, 1986; Joubès et al*.,* 2000; Lemaire-Chamley et al*.,* 2005). The mesocarp, the intermediate layer, is a parenchymatous tissue formed by big cells with large vacuoles (Joubès et al*.,* 2000; Lemaire-Chamley et al*.,* 2005; Mintz-Oron et al*.,* 2008). The cells of the mesocarp commonly undergo six to eight rounds of DNA duplication (endocycles) reaching ploidy levels of up to 512C (Bourdon et al*.,* 2010) and are reminiscent of the palisade cells of leaves (Gillaspy et al*.,* 1993) since they contain several chloroplasts, the organelle where photosynthesis occurs and produces up to 20% of fruit photosynthates, whereas the rest of photo assimilates are imported from source leaves (Hetherington et al*.,* 1998).

Tomato fruit development can be classified into four different phases. The first, dubbed the fruit set phase, corresponds to the stage where the development of the ovary either proceeds or aborts. The initiation of ovary development normally depends on the success of the pollination and fertilization. The ovary begins to develop into the fruit once the ovules are fertilized. During this stage, cell division and enlargement result in slow growth. Plant hormones such as gibberellins and auxins have been shown to play an important role in fruit set. These hormones when artificially applied can trigger the development of parthenocarpic (seedless) fruits (Gustafson, 1960; Gillaspy et al*.,* 1993). Again, this phase is described by rapid cell division, thereby resulting into a progressive increase in pericarp cell number.

The rate of cell division decreases barely two weeks after pollination marking the end of phase one. At this stage the fruit is about 1 cm in diameter. The subsequent phase of fruit development is characterized by a period of extensive cell division of the fruit tissue that generally takes between seven and ten days after fertilization depending on genotype (Varga and Bruinsma, 1966; Mapelli and Lombardi, 1982; Bohner and Bangerth, 1988). All through this stage, fruit growth depend on cell expansion and leads to a significant increase in weight. Cell expansion coincides with endo reduplication (Bergervoet et al*.*, 1996). By the end of this stage fruits have a diameter of around 2 cm. Throughout the third phase, the fruit enters the mature green (MG) stage (Ho and Hewitt, 1986; Giovannoni, 2004; Czerednik et al*.,* 2012) and attains its final size, which varies greatly among cultivars and is very susceptible to environmental influences (Chevalier, 2007). Most fruit growth is associated with the subsequent expansion phase during which cells undergo substantial endoreplication resulting in the production of large high ploidy cells with a diameter of more than 0.5 mm and up to 512C DNA content (Cheniclet et al*.,* 2005). This growth phase is driven by the accumulation of water in the vacuole. At the end of the cell expansion period, the tomato fruit reaches its final size and contains mature seeds. Roughly two days after reaching the mature green (MG) stage, the tomato fruit undergoes an extensive metabolic reorganization, which marks the beginning of the fruit ripening process (Ho and Hewitt, 1986). The mature fruit then undergoes the last phase of fruit development, known as ripening. Two main phases can be distinguished, which are referred to as the breaking (BR) and the ripening (RR) stages. From a botanical point of view, the tomato fruit is a berry, which can be bi- or multilocular. The septa of the carpels divide the ovary and the fruit into two or more locules. Seeds develop attached to the placenta, a parenchymatous tissue, which becomes gelatinous and fills the locular cavities during fruit development and maturation (Grierson and Kader, 1986; Ho and Hewitt, 1986; Bertin, 2005; Mintz-Oron et al*.,* 2008).

TOMATO FRUIT RIPENING AND RIPENING MUTANTS

Ripening is a phenomenon accountable for producing fruit that are eye-catching for consumption. Ripe fruits are a great source of energy, minerals, vitamins, carotene and antioxidants. Ripening is studied not only for its role in nutrition, but also its unique and complex developmental process requiring the well-coordinated regulation of numerous biochemical pathways. Study of fruit ripening is therefore valuable not only for practical agricultural purposes but also to better understand the regulation and transposition of plant developmental programs. Ripening is said to be a deteriorating process involving senescence and the general breakdown of cells. Fruit ripening is a sophisticatedly orchestrated developmental process, unique to plants, that results in major physiological and metabolic changes, ultimately leading to fruit decay and seed dispersal. Because of their strong impact on fruit nutritional and sensory qualities, the ripening associated changes have been a matter of sustained investigation aiming at unraveling the molecular and genetic basis of fruit ripening. All biochemical, molecular, physiological and structural modifications associated with ripening are tightly orchestrated at the genetic level, enabling the control of appearance, aroma and flavour. Ethylene is required for

fruit ripening and plays other important roles in plant growth and development (Barry, 2007). Ripening in the cultivated tomato comprises a series of biochemical and physiological events, including softening, pigment change, development of flavor components, autocatalytic ethylene production, and climacteric respiratory behavior, which together make ripe fruits. During ripening, tomato increases their ethylene level and subsequently undergoes various physiological changes. These changes occur simultaneously and are caused by the highly synchronized expression of numerous genes at the onset of ripening. Ethylene is rapidly produced at the breaker stage of the fruit and drives a series of reactions that together define the fruit ripening process. Generally, ripening involves softening of the fruit tissue, conversion of starch to sugar, accumulation of secondary metabolites affecting appearance, taste and aroma (Seymour, 1993). Polygalacturonase (PG) is the most important softening enzyme in tomato. It is absent in green fruit and accumulates in large quantities during ripening. The presence of this enzyme is correlated with the onset of cell wall degradation. Natural ethylene synthesis begins before PG appears and exogenous ethylene causes the accumulation of this enzyme in mature fruit (Grierson and Kader, 1986).

The process that leads to tomato ripening can be further divided into mature green stage, breaker stage and red ripe fruit stage. The mature green stage refers to the final-sized fruit containing mature seeds before initiation of ripening. The breaker stage corresponds to the beginning of the ripening program in the fruit, and is characterized by the first visual sign of ripening (orange color at the base of the fruit) and production of high levels of ethylene. Numerous genes associated with ripening begin to be expressed at high level at this stage (Alba et al*.,* 2004). The red ripe fruit stage corresponds to the fruit having completed the ripening program. Again the time required to reach red ripe fruits from BK is typically three to ten days, depending on the variety.

Although numerous ripening-associated traits have been shown to be influenced by ethylene, the identification and characterization of several naturally occurring ripening mutations have revealed another layer of regulation acting upstream of ethylene. Ripening mutants particularly alcobaca (*alc*), non-ripening (*nor*), never ripe (*nr*) and ripening inhibitor (*rin*) have gained a considerable attention in the last few years to prolong shelf life of tomato. These genes generally inhibit ethylene synthesis and or modify ethylene's downstream effects on specific biochemical processes related to fruit ripening. The fruits of these mutants are generally characterized by an absence of a ripening-associated ethylene burst, and an inability to ripen in the presence of exogenous ethylene. They are useful in research and breeding of cultivated tomatoes for postharvest quality. Kopeliovitch et al. (1979) have used several ripening gene mutants such as *alc*, *nor*, *nr* and *rin* to develop lines and cultivars with delayed ripening through disruption of the ethylene signalling pathway. Rodriguez et al. (2010) also explored the inheritance of fruit quality traits such as fruit shelf life in some tomato crosses using an Argentinean cultivar and a ripening mutant (*nor*). The mutant alleles *rin*, *nor* and *alc* generate a somewhat similar extended shelf life phenotype in heterozygous plants at these loci and have different modes of action.

CHARACTERISTIC EXPRESSION AND EFFECTS OF *rin, nor* AND *alc*

Ripening inhibitor (*rin*) is a regulator of ripening that directly influences many ripening- associated processes in a specific pattern. The *rin* mutant appeared in an F4 breeding line developed by H. M. Munger at Cornell University. The recessive gene altered several aspects of ripening as fruits did not ripen fully, turned yellow and softened very slowly (Robinson and Tomes, 1968). It encodes a genetic regulatory component necessary to trigger climacteric respiration and ripening-related ethylene biosynthesis. The *rin* gene is located on tomato chromosome 5 and belongs to the MADSBOX family of transcription regulators which is known to play essential roles in a variety of plant development processes (Riechmann and Meyerowitz, 1997). The name MADS is an abbreviation of the four founding members of the family: Mcm1 from *S. cerevisiea* (Passmore et al*.,* 1989), AGAMOUS from *Arabidopsis thaliana* (Yanofsky et al*.,* 1990), DEFICIENS from *A. majus* (Sommer et al*.,* 2000) and Serum Response Factor (SRF) from Homo sapiens (Norman et al*.,* 1988). MADS box genes encode DNA-binding proteins involved in many developmental processes in yeast, insects, nematodes, lower vertebrates, mammals and plants (Becker and Theissen, 2003; Messenguy and Dubois, 2003). In this regard, *rin* is a comprehensive ripening regulator explaining both the severe ripening inhibition of the mutation and its utility in coordinately slowing virtually all ripening processes in hybrid *Rin*/*rin* fruit predominant in current fresh market tomato production. Tomato plants homozygous for the ripening-inhibitor (*rin*) mutation have fruits that fail to ripen. Moreover, *rin* plants show enlarged sepals and loss of inflorescence determinacy. Two tandem MADS-box genes (LeMADS-RIN and LeMADS-MC) were revealed at positional cloning of the *rin* locus. Their expression patterns suggested roles in fruit ripening and sepal development for LeMADS-RIN and LeMADS-MC respectively. The *rin* mutation alters expression of both genes. The *rin* mutant exhibits ethylene sensitivity, including the seedling triple response, floral abscission, and petal and leaf senescence. Nevertheless, *rin* fruit do not ripen in response to exogenous ethylene, yet they display induction of at least some ethylene responsive genes, indicating retention of fruit ethylene sensitivity. The *rin/rin* homozygous plant produces fruit that never

fully ripen and have much firmer fruit with a significantly longer shelf life. At maturity, fruits are green and later turned into bright yellow, retarded ripening.

In the heterozygous condition *rin*/+ plants produce fruit with near normal fruit colour and flavour, and shelf life that is intermediate between *rin/rin* and +/+ (wild type) plants. *Rin/rin* lines generally have very low fruit sugars. They however, vary in sugar levels between *rin/rin* lines and +/+ lines. Lycopene levels in rin/+ hybrids is a little lower than wild type. Ripening is a little slower with *rin*/+ hybrids. Tomatoes heterozygous for the *rin* allele stay firm and ripen over a lengthened period. This is most probably due to reduced levels of functional RIN protein which allows industrial-scale handling and expanded delivery and storage opportunities.

The non-ripening (*nor*) gene was identified in an introduction, 'Italian Winter,' by E.A. Kerr at Horticultural Research Institute, Ontario. The *nor* gene is located on tomato chromosome 10 and belongs to the NAC family of transcription factors whose members play important regulatory roles in numerous developmental programs (pathways) (Olsen et al*.,* 2005). The NAC family is the largest plant-specific family of transcription factors with more than 100 members identified in *A. thaliana* (Riechmann et al*.,* 2000). NAC genes are named for their 18 founding members*:* Petunia *hybrida* NAM and *A. thaliana* ATAF1/2 and CUC2 genes (Souer et al*.,* 1996; Aida et al*.,* 1997). The *nor* gene is an unrelated transcription factor that also serves as a master regulator of fruit ripening in tomato. The *nor/nor* homozygous plant has a very similar phenotype to *rin/rin,* and *nor*/+ hybrids also have much restored color and flavor with extended shelf-life. *Nor* prevents normal fruit ripe (non-pigmentation, non-softening and crack resistance in fruits). This mutation is often used in the heterozygous form to create long shelf life of fruits. The non-ripening phenotype associated with the *nor* mutation suggests that many biochemical pathways are influenced by this gene.

The cultivar Alcobaca from Portugal possessing slow ripening gene *alc* was discovered in the 1960'sdescribed as a tomato with prolonged fruit storage life (Almeida, 1961) and potato type leaf (Lu et al*.,* 1995, Robinson and Tomes, 1968). Alcobaca genes in its homozygous form is noted to obstruct or significantly slow down a wide range of processes associated with ripening of tomato fruit leading to a noticeably extended shelf life (Kopeliovitch et al*.,* 1979; Lobo et al., 1984; Mutschler, 1984b; Tigchelaar and Rios, 1989; Lu et al*.,* 1995; Ignatova et al., 1999; Dhatt, 2001; Garg, 2006) but inferior flavour (Kopeliovitch et al*.,* 1980, 1982) and poor colour development (Sink et al*.,* 1974; Kopeliovitch et al*.,* 1980; Lobo et al*.,* 1984). However, in plants heterozygous for these alleles, several ripening characteristics exhibit levels intermediate between those of the wild and mutant parents and produce fruits having shelf life many times greater than normal cultivars (Kopeliovitch et al*.,* 1979; Mutschler et al*.,* 1992; Dhatt, 2001; Kitagawa et al., 2005; Garg, 2006), besides developing acceptable colour (Kopelivitch et al*.,* 1981; Mutschler, 1984b; Gavrish and Korol, 1991) and flavour attributes (Kopeliovitch et al*.,* 1982; Agar et al*.,* 1994). This semi-climacteric mutant causes a ripening syndrome characterized by attenuated respiratory activities and ethylene production, delayed softening of the fruit, low PG activity and extended shelf life (Mutschler, 1984b). Kopeliovitch et al. (1980) found that the final colour of mutant fruits picked at mature green stage, breaker stage and two weeks post breaker stage was yellow, orange and light red, respectively.

UTILIZATION OF *rin, nor* AND *alc* ALLELES

Fruits of tomato are only available for a short time. Excess production of tomato during these short periods leads to a saturated market and low prices. These periods can be followed by scarcity and high prices. Mutant genes such as alcobaca (*alc*), ripening inhibitor (*rin*), and non-ripening (*nor*) in heterozygous form extend shelf-life and take more time to go from mature green to red ripe as compared to normal genotypes (Buescher et al*.,* 1976; Kopeliovitch et al*.,* 1979; McGlasson et al*.,* 1983; Nguyen et al., 1991; Mutschler et al*.,* 1992; Agar et al*.,* 1994; Lu et al*.,* 1995; Dhatt, 2001; Kitagawa et al*.,* 2005) and develop acceptable color (Sink et al*.,* 1974; Ng and Tigchelaar, 1977; Hobson, 1980; Kopeliovitch et al*.,* 1981; Mutschler, 1984; Lu et al*.,*1994) and flavor attributes (Kopeliovitch et al*.,* 1982; Agar et al*.,* 1994). Fruit of F1 hybrids carrying these mutant alleles take longer to transition from mature green to red ripe stages as compared to normal genotypes (McGlasson et al*.,* 1983; Nguyen et al*.,* 1991; Dhatt, 2001). Sinha et al. (2014), in their work on tomato shelf life, evaluated twenty four F7 RIL'S developed from the cross L121 X Vaibhav for high shelf life. L121 is a parent having *alc* gene is able to prolong shelf-life but has poor agronomic character. The evaluation of F7 RILs resulted in the identification of tomato RILs with high shelf life. Among F7 RILs, parents and checks, the maximum shelf life was observed in RIL 7-3, RIL 110-2 (110 days), followed by RIL 102-1 and RIL 182-4 (100 days) with an average shelf life of the F7 RILs were 63 days.

According to Giovannoni (2007), the discoveries of fruit ripening mutants have yielded insights into ripening control. He further mentioned that these findings have produced new understanding of the primary ripening control mechanisms which include transcription factors such as those encoded by the RIPENING- INHIBITOR (RIN) MADS-box and COLOURLESS NON-RIPENING (CNR) SPB-box genes. These are actually necessary for the progression of virtually all ripening processes. They have also facilitated the elucidation of downstream signal transduction components that impact the hormonal and environmental stimuli that coordinate and modulate ripening phenotypes. He demonstrated in his report that

physiologically characterized single gene tomato ripening mutants, which in some cases have been available for decades, have recently become accessible and useful at the molecular level as the genomics infrastructure for tomato has expanded.

Lavy-Meir et al. (1989) also reported that tomato ripening mutants and their hybrids showed resistance to *Botrytis cinerea.* Their work examined the resistance of mutant fruits to infection by *B. cinerea,* one of the main pathogens of tomato fruit by studying differences in conidia germination, infection and lesion development among normal, mutant and hybrid fruits. Yasuhiro (2016) further confirmed in his report or review that the identification of ripening mutants particularly key regulatory gene *rin* in tomato has opened new horizons in our understanding of fruit ripening. He also reported that the *rin* mutant removes the transcriptional activation activity from the protein thereby preventing the up-regulation of genes required for ripening (Ito et al*.,* 2008). Additionally, his review revealed that *rin* regulates other transcription factors involved in fruit ripening, including *nor* and *cnr*. Ethylene mediates the induction of ripening-associated genes under the control of the ripening regulating transcription factors, and the ethylene signalling pathway also enhances the expression of RIN, FUL1, and NOR in a positive feedback loop (Fujisawa et al*.,* 2013). Understanding of ripening regulation, especially the crucial role of MADS-box transcription factors, have expanded scientists ability to control ripening-associated metabolic characteristics, thereby providing breeding methods for fruits with better taste, aroma, nutrition, and shelf life. The author further reported that apart from biological interest in ripening regulation, breeding programs have targeted the RIN locus because the RIN/rin heterozygous genotype produces red-ripe fruits with a remarkably extended shelf life (Kitagawa et al*.*, 2005). The heterozygous genotype has also been proposed for use in developing tomatoes with low levels of allergens (Kitagawa et al*.,* 2006).

PROBLEMS AND PROSPECTS OF TOMATO RIPENING MUTANTS

The remarkable features of *rin, nor* and *alc* mutants are their extended shelf life beside quality parameters (except colour) comparable with the normal fruit (Kopeliovitch et al., 1979; MutscWer et al., 1984b; Lu et al., 1994; Hobson 1980; McGlasson, 1983; Lobo et al., 1984; Lobo et al., 1991; Mutschler et al., 1992). Mutant genes such as *rin, nor* and *alc* can be used to extend the shelf life of tomato (Leal and Tabim, 1974; Robinson and Tornes, 1968; Tigchelaar et al*.,* 1973). The fruits of these mutants are elite by an absence of a ripening-associated ethylene burst, and an inability to ripen in the presence of exogenous ethylene. This phenotype is described as a failure to reach ripening competency, a developmentally regulated stage in which a fruit becomes responsive to ethylene. These mutant genes not only delay the normal process of ripening but also have undesirable pleiotropic effects on other components of fruit quality (Kovacs et al*.*, 2009; Matas et al*.,* 2009; Mutschler et al*.,* 1992; Thompson et al*.,* 1999; Tigchelaar et al*.,* 1978). The abnormal pattern of fruit colour development in the homozygous form has restricted their use at commercial levels (Robinson and Tomes, 1968; Tigchelaar et al*.,* 1973; Lobo et al*.,* 1984). For instance, the non-ripening (*nor*) gene repress the normal ripening of tomato fruit. The molecular mechanism of fruit ripening regulation by the *nor* gene is however, unclear. Unfortunately, in homozygous state these genes do not develop normal colour (Kopeliovitch et al*.,* 1979). Even exogenous application of ethylene is not helpful in stimulating colour development (Tigchelaar et al*.,* 1978). Some efforts employed by some scientists to overcome the problem associated with the homozygous state of the ripening mutant genes is by cross combinations with desired genotypes for colour improvement. Trinklein and Lambeth (1976) proposed incorporation of uniform ripening and colour enhancing genes to improve colour.

Nguyen et al. (1991) and Dhatt (2001) demonstrated that *rin, nor* and *alc* alleles in heterozygote form nonetheless, developed acceptable colour for marketing. This was also confirmed by Mutschler (1984), Lu et al. (1994) and Seroczynska et al. (1998) who reported that the fruits of hybrids between normal homozygotes (+/+) and mutant homozygotes (*nor/nor, rin/rin and alc/alc*) do ripen naturally and exhibited extended shelf life too. Hence, this offers an opportunity to exploit these genes to regulate tomato supply for longer period. The commercial use of *rin, nor* and *alc* mutants has been made in many countries. In Australia, several varieties have been developed using ripening mutants. For instance, 'Red Centre' (HRAS 87-70 x *rin-HRAS* 81-85) and 'Juliette' (79T-I x *rin-795054-1)* hybrids having 40 days shelf life at 20°C temperature have been released (Nguyen et al*.,* 1991; Nguyen 1994). The *nor* gene has been exploited by Gavrish and Bogdanov (1992) in Russia by developing 'Vasilisa' hybrid, 'Changline' an outstanding *nor* hybrid with exceptional shelf life was also released in China (Lu et al*.,* 1994). The F1 between S 15 x *110r* was registered as Rafal' in Poland, which has storage life of 77 to 99 days. It also provided 2 to 4 weeks delay in last harvest along with high firmness, acceptable colour and good quality (Seroczynska et al*.,* 1998). Likewise in the USA (Suslow and Cantwell, 1997) and India, ripening mutant genes have been successful at the commercial level (Dhatt, 2001). The alcobaca (*alc*) gene, a mutant involved in the ripening process of tomato plant fruits, have allowed an understanding about some effects that it causes on the quality and post-harvest conservation traits of tomatoes. The effects of the *alc* allele on several plant and fruit traits were studied by Mutschler et al. (1992), Flori (1993), Flori and Maluf (1994), Resende (1995),

Souza (1995) and Freitas (1996).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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