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SHORT COMMUNICATION

Impacts of *Sletr1-1* and *Sletr1-2* mutations on the hybrid seed quality of tomatoes

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Abstract

Shelf life is an important breeding trait in tomato, especially for the tomato production in subtropical and tropical regions. Previously we have isolated and characterized ethylene receptor mutants, *Sletr1-1* and *Sletr1-2* from mutant population based on Micro-Tom cultivar. *Sletr1-1* showed insensitivity to ethylene while *Sletr1-2* showed reduced sensitivity to ethylene. We also have demonstrated that the traits are useful for extending fruit shelf life of the hybrid tomato cultivars. For commercializing the hybrid cultivars, the seed quality is another important trait. In this study, we evaluated the effects of the *Sletr1-1* and *Sletr1-2* mutations on the seed quality characteristics of F_1 hybrid lines generated by crossing *Sletr1-1* and *Sletr1-2* with three commercial tomato cultivars, Intan, Mutiara and Ratna. *Sletr1-1* mutation conferred insensitivity to ethylene in the F_1 hybrid seedlings, resulting in negative effects including reduced germination rate, vigor index and emergence speed index. Interestingly *Sletr1-2* mutation had almost no effect on the seed quality characteristics of the F_1 hybrid lines, suggesting that *Sletr1-2* was suitable for producing high quality of hybrid seeds.

Keywords: ethylene receptor, mutant, hybrid quality seed, tomato

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the important horticultural crops in the tropics and subtropics, because it contains high levels of micro- and macro-nutrients such as vitamins, minerals, fiber and other compounds desirable for human health. Since one of the major concerns in tomato production is a postharvest loss of fruits, considerable efforts have been made on breeding new tomato cultivars conferring long shelf life for the fruits. Both natural and induced mutants giving extended fruit shelf life were used in the breeding programs. Several genetic mutants affecting tomato ripening, such as *ripening-inhibitor* (*rin*), *Colorless non-ripening* (*Cnr*), *non-ripening* (*nor*), *Never-ripe* (*Nr*), *Sletr1-1* and *Sletr1-2* have been isolated and characterized (Lanahan *et al*. 1994; Wilkinson *et al*. 1995; Vrebalov *et al*. 2002; Okabe *et al*. 2011). *Sletr1-1* and *Sletr1-2* mutations could significantly extend the shelf life of tomato fruits, and *Sletr1-2* mutation especially conferred prolonged fruit shelf life without a reduction in carotenoid content (Okabe *et al*. 2011). Subsequently, we demonstrated the use of *Sletr1-1*

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and *Sletr1-2* mutations in hybrid tomato breeding; *Sletr1-2* mutation can extend fruit self life in the F_1 hybrid lines with minimal effects on the agronomic traits (Mubarok *et al*. 2015, 2016).

Seed quality is another important breeding trait that is important for commercializing hybrid seeds. Since the 1920s, many studies have shown that ethylene regulated seed quality by affecting the seed germination (Egley 1982). At present, it is an established fact that in many species, the application of ethylene or the ethylene-releasing compound, breaks primary dormancy, secondary dormancy in seeds and accelerates the germination of non-dormant seeds (Kepczynski 1985; Esashi 1991). Other studies have shown that ethylene is involved in enhancing seed dormancy, vigor and the time required for radicle emergence as a measure of seed vigor (AOSA 1983; Khan 1994). The evidence of the reduction of seed quality in an ethylene-insensitive mutant was observed in the *Nr* mutant that affects seed vigor (Siriwitayawan 2003). Numerous studies showed that a reduced ethylene production in chickpea, sunflower and lettuce is associated with the induction of thermodormancy (Corbineau *et al*. 1988; Prusinski and Khan 1990; Gallardo *et al*. 1991). Therefore, when using ripening-related mutants in a breeding program selecting for long shelf life, the effects of the mutations on seed quality characteristics should be carefully evaluated.

Consequently, when generating new F_1 hybrid cultivars with a long shelf life using the *Sletr1-1* and *Sletr1-2* mutations, the effects of these mutations should be clearly evaluated. In this study, we evaluated the effects of the *Sletr1-1* and *Sletr1-2* mutations on the seed quality characteristics of F_1 hybrid lines generated by crossing *Sletr1-1* and *Sletr1-2* with three commercial tomato cultivars, Intan, Mutiara and Ratna.

2. Materials and methods

2.1. Plant materials and growth conditions

Sletr1-1 and *Sletr1-2* are the mutants, which isolated and characterized from a mutant population based on Micro-Tom cultivars (Okabe *et al*. 2011). *Sletr1-1* showed insensitivity to ethylene whereas *Sletr1-2* showed reduced sensitivity to ethylene. The varieties Intan, Mutiara, and Ratna are the commercial pure-line cultivars, which were generated by the Vegetable Crops Research Institute, Indonesia. Those three cultivars are beef tomatoes that are adaptive for the Indonesian climate. Those plants were cultivated under normal condition using a combination of growing media of soil+compost+peat moss (1:1:1, v/v) in the greenhouse from August to November 2017, with a day temperature of 33°C and night temperature of 22°C. The hybrid seeds were generated by crossing these mutants, *Sletr1-1* and *Sletr1-2*, and the wild-type Micro-Tom (WT-MT) as a pollen donor, with the three pure-line cultivars, Intan, Mutiara and Ratna, as a pollen recipient. The hybrid F_1 seeds were harvested from the red fruit of the cross between the ethylene receptor mutants and pure-lines cultivars. Harvested seeds were rinsed with water to remove the gel around the seed, and then they were dried at room temperature. After that, the dried seeds were kept in storage at 4°C.

2.2. Seed quality analysis

To analyze the seed quality of mutant and hybrid lines, several parameters including seed germination, emergence speed index and vigor index have been investigated. Seed analysis was conducted based on the ISTA analysis protocol for seed testing (ISTA 2017). In brief, 400 seeds were germinated in wet paper using the top paper method and this was repeated four times. They were distinguished and counted for normal seed characterized by the root and shoot being well developed and abnormal seed characterized by the root or shoot being not well grown, the structure being deformed or disproportionate and otherwise improperly developed. Germination rate was counted based on the percentage of normal seed at 5 and at 7 days after showing (DAS), respectively, using the following equation:

$$\text{Germination rate (\%)} = \frac{\sum\text{Normal seed at 5 DAS} + \sum\text{Normal seed at 7 DAS}}{\sum\text{Total investigated seed}}$$

Vigor index was calculated as a percentage of normal seed that was counted at 5 DAS, whereas emergence speed index was counted daily for 14 days and calculated according to Maguire (1962).

2.3. Triple response assay to ethylene

The triple response assay was conducted to investigate the ethylene sensitivity of F_1 hybrid lines according to the method of Mubarok *et al*. (2015). Briefly seeds were sterilized for 20 min and grown in a 50-mL glass bottle sealed with rubber septum and containing 10 mL of 1/2 Murashige and Skoog (MS) medium (Murashige and Skoog 1962). Total of 5 μL L^{-1} of exogenous ethylene was injected into the sealed glass bottles containing seeds and kept in the dark at 25°C for 7 days.

2.4. Statistical analysis

A completely randomized design was used for this experiment. The data were represented as the mean

value±SE of four replicates. For the statistical data analysis, data were subjected to Student's *t*-tests at $P<0.05$ for comparisons between the investigated data and the control.

3. Results

Under homozygous condition, the reduction of seed quality was detected in *Sletr1-1* and *Sletr1-2* mutant alleles. Reduction of germination rate of *Sletr1-1* and *Sletr1-2* was 20 and 16% lower than WT-MT, and also for emergence speed index was 48 and 43% lower than WT-MT for *Sletr1-1* and *Sletr1-2*, respectively. For vigor index, mutation in *Sletr1-2* showed the comparable effect compared with WT-MT, but mutation in *Sletr1-1* significantly decrease the vigor index (Fig. 1). Improving the seed quality as an effect of *Sletr1-2* mutation was detected in heterozygous lines of *Sletr1-2* F1, but the effect of *Sletr1-1* mutation in improving the seed quality did not detect in *Sletr1-1* F1 (Fig. 2). *Sletr1-1* F_1 seeds exhibited a reduction in the germination rate compared to WT-MT F_1 seeds in all the parental backgrounds, Intan, Mutiara and Ratna (Fig. 3-A). Based on this result, the *Sletr1-1* mutation gave a similar response on the reduction of germination rate for several different genetic backgrounds. The data showed that the lowest germination rate was detected in the hybrid line of *Sletr1-1*×Intan, 29.5% lower than WT-MT F_1 hybrid seeds. On the other hand, it can be seen that the reduction of germination rate did not occur in *Sletr1-2* F_1 seeds. Even when the *Sletr1-2* was crossed with varieties having a different genetic background, the germination rate was not changed compared to WT-MT F_1 seeds.

The negative impact of the *Sletr1-1* mutation was also observed in the reduction of seed vigor index and emergence speed index. However, the negative impact was not detected in the *Sletr1-2* F_1 hybrid seeds from all parental backgrounds (Fig. 3). The reduction of vigor index was significantly detected in *Sletr1-1* F_1 seed with a parental background of Intan and Mutiara, but it was not detected under the Ratna parental background. The vigor index of *Sletr1-1* F_1 hybrid from Intan and Mutiara parental backgrounds was 3.5 and 1.5 times lower than the vigor index of WT-MT F_1 seeds, respectively. The reduction of emergence speed index of hybrid seeds as an effect of *Sletr1-1* mutation was detected in the F_1 lines from all parental backgrounds. The emergence speed index of *Sletr1-1* F_1 hybrid seeds was 7.36, 9.27, and 8.99 or 6.44, 5.14, and 4.23 lower than WT-MT F_1 hybrid seeds for the parental background of Intan, Mutiara and Ratna.

A reduction of ethylene sensitivity was observed in all of the *Sletr1-1* F_1 and *Sletr1-2* F_1 hybrid lines by increasing the length of shoot and roots on seedlings under 5 μL L^{-1} of exogenous ethylene exposure. *Sletr1-1* conferred

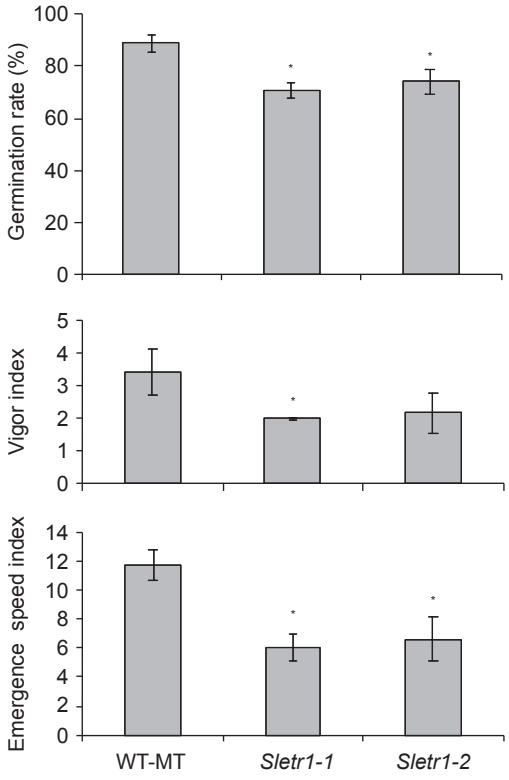

Fig. 1 Impact of *Sletr1-1* and *Sletr1-2* mutations on the seed quality (germination rate, vigor index and emergence speed index). WT-MT, wild-type Micro-Tom. The mean value±SE (four replicates) followed by the asterisk are significantly different from the control (WT-MT) based on Student's *t*-tests at $P<0.05$.

insensitivity to the seedlings of those hybrid lines, whereas *Sletr1-2* conferred reduced sensitivity to the seedlings of those hybrid lines. Under 5 μL L^{-1} of exogenous ethylene, hypocotyl and shoot length of all of *Sletr1-1* F_1 hybrid lines significantly longer than WT-MT F_1 hybrid lines. The shoot length of *Sletr1-1* F_1 hybrid lines were 7.07, 6.51 and 6.5 cm longer than WT-MT F_1 hybrid line under a parental background of Intan, Ratna and Mutiara (Fig. 4-A). Whereas, the root length of *Sletr1-2* F_1 hybrid line were 2.92, 3.27 and 3.23 cm longer than WT-MT F_1 hybrid line under parental background of Intan, Mutiara and Ratna (Fig. 4-B).

4. Discussion

It has been proposed that the *Sletr1* mutant alleles (*Sletr1-1* and *Sletr1-2*) and *Sletr1* mutant allele (*Sletr1-4*) are potential breeding materials for extending shelf life of tomato fruits (Okabe *et al*. 2011; Mubarok *et al*. 2019). Commercialization of new hybrid lines using *Sletr1-1* or *Sletr1-2* would contribute to reducing the postharvest loss of tomato fruits. *Sletr1-1* and *Sletr1-2* have a mutation in

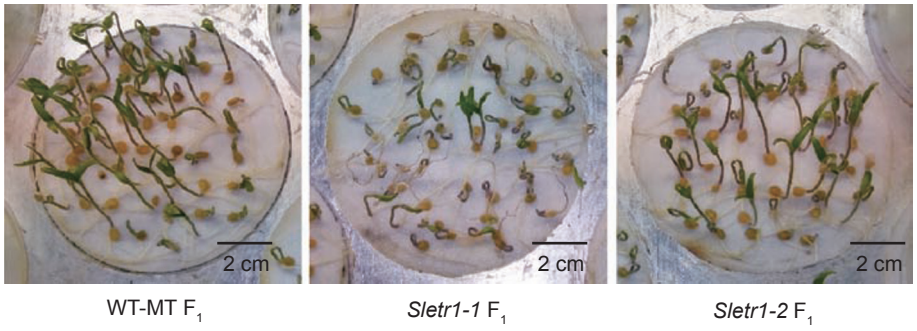

Fig. 2 Representative pictures for seed growth of WT-MT F_1, *Sletr1-1* F_1 and *Sletr1-2* F_1 hybrid lines crossed with parental background of Intan. WT-MT, wild-type Micro-Tom.

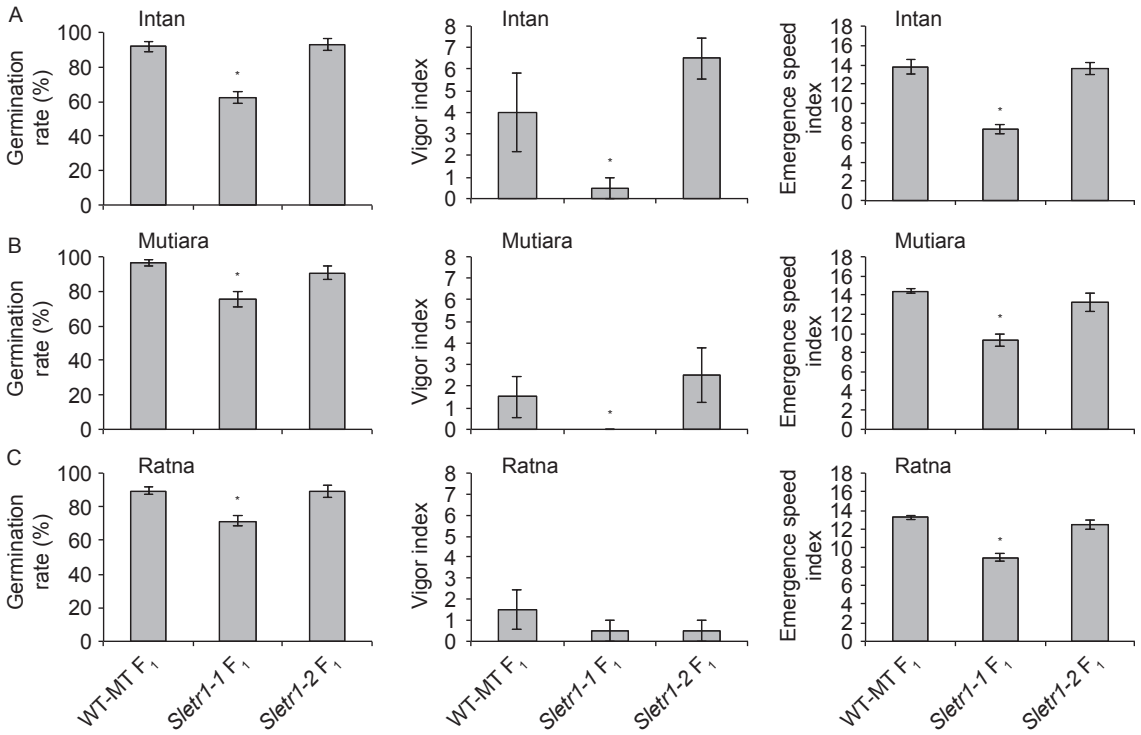

Fig. 3 Impact of *Sletr1-1* and *Sletr1-2* mutation on the seed quality (germination rate, vigor index and emergence speed index) of F_1 hybrid lines in different parental backgrounds, Intan, Mutiara, and Ratna. WT-MT, wild-type Micro-Tom. The mean value±SE (four replicates) followed by the asterisk are significantly different from the control (WT-MT F_1 line) based on Student's *t*-tests at $P<0.05$.

the first and second transmembrane domain of the ethylene receptor gene (*SlETR1*), respectively; therefore the effect of ethylene can be minimized and finally the fruit shelf life was increased (Okabe *et al*. 2011; Mubarok *et al*. 2015). However, it is speculated that its mutation might affect the seed quality, which is another important breeding trait in tomato. Therefore this study evaluated the effects of *Sletr1-1* and *Sletr1-2* mutations on the hybrid seed quality.

High quality seeds are required for the commercialization of hybrid seeds. Several parameters are used to determine the seed quality such as seed germination, seed vigor and emergence speed index. Numerous studies demonstrate that ethylene production has a correlation with the seed germination, therefore suggesting the involvement of ethylene in the regulation of seed development, germination and dormancy (Matilla 2000). Corbineau *et al*. (2014) stated that the application of exogenous ethylene improves germination in numerous species and stimulates the germination under non-optimal environmental conditions. Inhibition of ethylene action and response by the mutation of ethylene receptor gene resulted in a different seed quality characteristics depend on the mutation location in *SlETR1* gene. Undesirable effects of *Sletr1-1* mutation compared to the control were observed in the *Sletr1-1* F_1 hybrid seeds

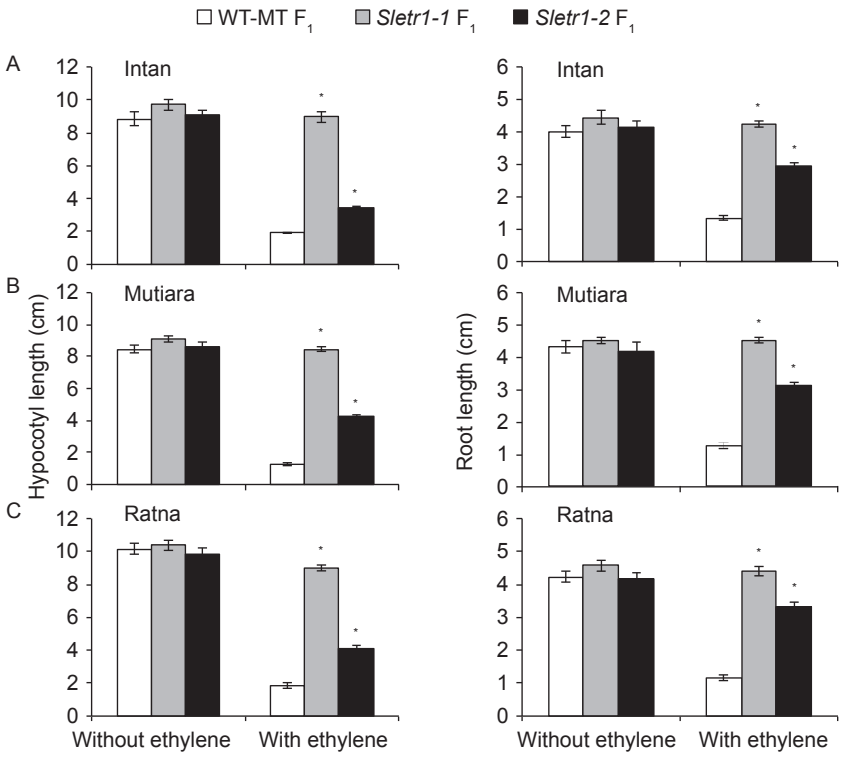

Fig. 4 Ethylene sensitivity of *Sletr1-1* and *Sletr1-2* F_1 hybrid lines under 5 μL L^{-1} of exogenous ethylene exposure. WT-MT, wild-type Micro-Tom. The mean value±SE (four replicates) in with or without ethylene treatment followed by asterisks is significantly different from the control (WT-MT F_1 line) based on Student's *t*-tests at $P<0.05$.

on different parental backgrounds. The *Sletr1-1* mutation significantly affects the reduction of seed germination, seed vigor and emergence speed index of the F_1 hybrid lines in all the different parental backgrounds, Intan, Mutiara and Ratna. However, these effects were not detected in the *Sletr1-2* F_1 hybrid lines (Fig. 3). *Sletr1-1* mutation conferred complete ethylene insensitivity in the F_1 hybrid lines. It was shown that the application of 5 μL L^{-1} ethylene did not affect the hypocotyl and primary root elongation. However, in *Sletr1-2* F_1 hybrid lines, ethylene slightly affects the reduction of hypocotyl and primary root elongation (Fig. 4), which correspond with our previous study (Mubarok *et al*. 2015). These results suggest that *Sletr1-1* and *Sletr1-2* mutations have the same phenotype effect on different parental background, which exhibited a dominant inheritance pattern.

Reduction in seed quality due to *Sletr1-1* mutation in this study is consistent with the *Nr* mutant seeds that exhibited a reduction in seed quality in Rutgers and Ailsa Craig parental backgrounds (Siriwitayawan *et al*. 2003). The same undesirable effects on seed quality for both mutations might be due to the same mutation location in ethylene receptor gene. Those mutants had a mutation located in the first transmembrane domains, which is an important region for ethylene binding. Amino acid substitution of P51L in the *Sletr1-1* mutant as a result of mutation in the first transmembrane domain is similar to the amino acid substitution of P36L in *Nr* (Mubarok *et al*. 2015). Another study showed a poor germination rate is also observed in the *Arabidopsis* mutant *etr1-1*, ethylene-insensitive mutants, which did not respond to exogenous ethylene (Bleecker and Schaller 1996; Bleecker *et al*. 1988). Based on these studies, we confirm that seeds that are strongly impaired for ethylene perception exhibited slower germination compared to wild-type seeds indicating reduced seed quality, whereas those only weakly impaired for ethylene perception exhibited almost similar germination to the wild-type seeds.

Abscisic acid (ABA) plays a role in the induction of dormancy and in the maintenance of dormancy during seed imbibition (Cutler *et al*. 2010; Nambara *et al*. 2010), whereas ethylene regulates seed germination and dormancy by decreasing sensitivity to endogenous ABA (Matilla 2000; Arc *et al*. 2013). Ethylene insensitivity led to a greater sensitivity to exogenous ABA and greater ABA biosynthesis (Beaudoin *et al*. 2000; Ghassemian *et al*. 2000). Inhibition of ethylene perception in *Sletr1-1* F_1 hybrid lines might affect the increasing ABA biosynthesis; therefore it affects the reduction of seed germination and vigor. Interestingly in *Sletr1-2* F_1 hybrid seeds, there was no clear reduction in seed quality. The germination rate of *Sletr1-2* F_1 hybrid lines seed relative to WT-MT F_1 hybrid seed might be

due to the *Sletr1-2* mutation being a moderate ethylene insensitive mutation that displays some limited ethylene perception (Okabe *et al*. 2011). Therefore, ethylene may still affect the seed germination and development. Several studies on tobacco seed showed that ethylene also acts in up-regulation of the gene for 1,3-glucanase, an enzyme participating in endosperm rupture (Leubner-Metzger *et al*. 1998; Leubner-Metzger and Meins 2000).

5. Conclusion

Sletr1-1 and *Sletr1-2* mutations resulted in the different effect on the seed quality of F_1 hybrid lines. Any undesirable effects of *Sletr1-2* mutation on the seed quality, i.e., germination rate, vigor index and emergence speed index have not been detected in *Sletr1-2* F_1 hybrid seeds. We conclude that the *Sletr1-2* mutant has a potential as a contributor to breeding programs for the new hybrid tomato cultivars with improved fruit shelf life.

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