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Full Length Research Paper

Gene expression and phenotypic characterization of flooding tolerance in tomato

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The effect of flooding on gene expression of four tomato genotypes was studied under a controlled environment. Four different flooding treatments (0, 2, 4, and 8 days) were used for flooding tolerance in four tomato genotypes. The experiment was laid out in a randomized split plot design with flooding treatment as main plot and the genotypes subplot. Treatments were replicated five times. The results show that with each flooding treatment, alcohol dehydrogenase (ADH) was up- regulated in genotypes CLN2498E and LA1579 compared to the control CA4. The activity of ADH was four times higher in LA1579 than in CLN2498E. In LA1579, as the days of continuous flooding increased, the sucrose synthase decreased. The death of LA1579 plants for 8 days of flooding might be due to the production of toxic substance. There was a significant difference ($P<0.05$) in plant height of CA4 and LA579 for 8 days of flooding. Although there was no difference in plant height of LA1421 but no fruits were recorded for 4 and 8 days of flooding because its plants dropped off their flowers. No significant difference in plant height of CLN2498E was recorded and it produced the highest yield ($P<0.05$). For these above reasons, CLN2498E and CA4 were the tolerant genotypes while LA1579 was the sensible genotype.

Key words: Alcohol dehydrogenase, sucrose synthase, tomato genotypes, flooding conditions.

INTRODUCTION

Flooding is among the environmental stressors that will increase with the change in climate. Environmental stresses (salinity, flooding, heat, drought, cold, etc.) have been a big challenge for plants to produce high yield (Ismond et al., 2003). The screening of plant genotypes which have prominent characteristics such as high tolerance to lack of oxygen is one way to contribute to the development of tolerance to flooding. Many plants exposed to flooding and oxygen deprived conditions cannot grow and will die within a few days (Harada et al., 2005). Many factors contribute to tolerance of plant crops to flooding stress; it is only in rice (*Oryza sativa*) that selection has been performed for flooding tolerance where a genetic mapping approach was used to identify major and minor genes involved in it (Sripongpangkul et al., 2000; Xu et al., 2000; Ismond et al., 2003). Tolerance in flooding involved the coordinated actions of several metabolic processes (Fagerstedt and Crawford, 1987) and alcohol dehydrogenase (ADH) activity increases in tomato during flooding stress (Tanksley and Jones, 1981), and contribute greatly to the survival of plants under

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Figure 1. Response of tomato variety CA4 to different days of flooding.

stress. Many studies reported that under flooding conditions, ADH increase its activities in many plants such as maize (Hageman and Flesher, 1960) and tomato (Tanksley and Jones, 1981).

Sato et al. (2002) reported that more sucrose is synthesized under anoxia than is synthesized in air; the content of sucrose in the stems of turions (it is resistant plant bud that is found in certain aquatic plants, and can allow plant to survive winter in the vegetative state) decreases rapidly within 1 day of anoxia, but remains constant thereafter. These results indicate that turnover of sucrose is activeted by anoxia. The activities of invertase, sucrose phosphate synthase and sucrose synthase have been shown to increase under anoxia and potentially the increase of this enzyme being especially notable (Harada and Ishizawa, 2003).

The aim of the present study was to investigate four tomato genotypes for flooding tolerance using the activity of ADH, sucrose synthase, and physiological traits.

MATERIALS AND METHODS

Seeds of four tomato genotypes: LA1579, CA4, CLN2498E, and LA1421 were used in this study and were obtained from the AVRDC gene bank. Seeds of these genotypes were sown in seedling trays filled with peat moss and watered daily. Growing plants were fertilized [fertilizer (Foliar Nitro-Phoska) containing nitrogen, phosphorus, potassium, magnesium (20-19-19-0.5)] at a rate of 2 g per pot at 3 weeks intervals. A mixture of pesticide (Pymetrozine 25% WP; Benlate 50% WP; Trigard 75% WP; Adjuvant and Chlorfuazuron 5% EC) was sprayed weekly to prevent the invasion of disease and insect infestation.

Experimental design and treatment details

The experiment was laid out in a randomized split plot design with flooding treatment as main plot and the genotypes subplot. Treatments were replicated five times and 16 plants were used per replication. 45 day old tomato plants were then subjected to flooding stress of different durations (0, 2, 4 and 8 days) by placing plant pots inside larger plastic pots, then irrigating with an excessive quantity of tap water at 25°C so that the level of water above the surface of soil was 15 cm throughout the flooding period. At the bottom of each plastic pot, a drilled hole allowed complete drainage of the pot after flooding. The plants growing in the greenhouse under normal non-flooded conditions served as a corresponding control. The experiment was conducted till reproductive stage.

Phenotypic measurements

On weekly basis, physiological parameters: plant height, number of leaves, leaf length and yellowing of leaves were measured.

Tissue collection

The leaves of the plants were collected in micro centrifuge tubes and immediately snapped frozen in liquid nitrogen. Tissue samples were stored at -80°C until required for extraction.

RNA isolation and cDNA synthesis

Total RNA was extracted from leaves with a GenMark Plant Total RNA Miniprep Kit (Hopegen Biotechnology) following the manufacturer's instructions. Total RNA was then treated with RNase-free DNase I to remove any genomic DNA contamination. RNA was then quantified using a Quibit fluorometer (Invitrogen, USA). First strand cDNA was synthesized using SuperScript III reverse transcriptase (Invitrogen) from 1 µg of total RNA in a 20 µl reaction. As a negative control, cDNA template was synthesized without SuperScript III reverse transcriptase.

Real time PCR reactions were performed with SYBRE Green Master Mix in a corbet6000 rotorgene; 6 µl of SYBRE Green and 0.8 µl primers to a final concentration of 15 µl. Cycling conditions were 45 cycles of : 95°C for 10 s, 60°C for 15 s, 72°C for 20 s. *Solanum lycopersicon* Actin (U60482) was used to normalize the genes. Primer sequences were as follows; Actin forward: aatgatcggaatggaagctg, actin reverse; atcctccgatccagacactg, sucrose synthase forward aagggtggccttaagcgtat sucrose synthase reverse, acagccaatgggacaagttc, alcohol dehydrogenase (ADH) forward, cctcgttcggatattccttg, AHD reverse, gtttagtccgccatggtgat. Primer PCR products were sequenced to confirm specificity. Three biological replicates were assayed for each genotype and each reaction was performed in duplicate. Quantifying the relative changes in gene expression was performed using $2^{-\Delta\Delta CT}$ method according to Livak and Schmittgen (2001) with the control plant CA4 as the calibrator (Figure 1).

Statistical analysis

The data collected were subjected to an analysis of variance using statistical analysis system (SAS) to determine the differences among treatments. Means separation was performed by Turkey's test.

RESULTS

Phenotypic analysis

There was no significant difference ($P<0.05$) in plant growth of tomato genotypes CLN2498E and LA1421 compared to their respective controls before during and after flooding (Figures 2, 3, 7 and 8). Significant difference

Figure 2. Response of tomato variety CLN2498E to different days of flooding.

Figure 3. Response of tomato genotype LA1421 to different days of flooding.

Figure 4. Response of tomato genotype LA1579 to different days of flooding

Figure 5. Wilting of (A) variety CA4 and (B) variety CLN2498E.

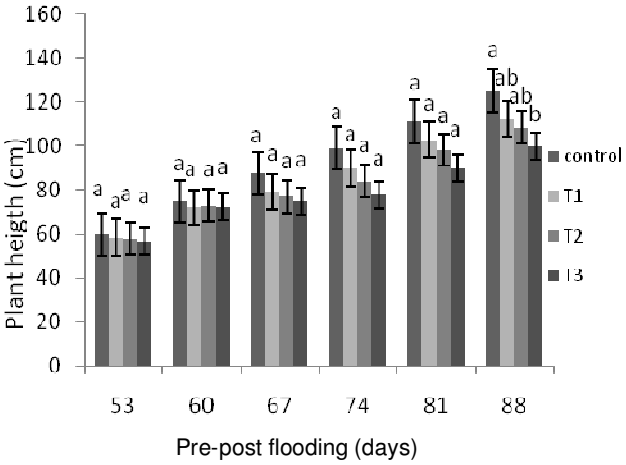

Figure 6. Plant height (cm) of CA4 grown under flooding conditions.

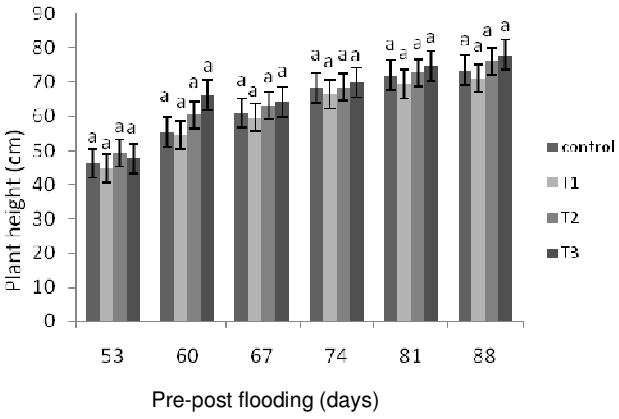

Figure 7. Plant height (cm) of CLN2498E grown under flooding conditions.

(P<0.05) was recorded in plant height of CA4 and LA1579 (Figures 1, 4, 5, 6 and 9).

Genotypes CA4 and CLN2498E (Figures 1 and 2) showed no sign of leaf senescence; the leaves from these

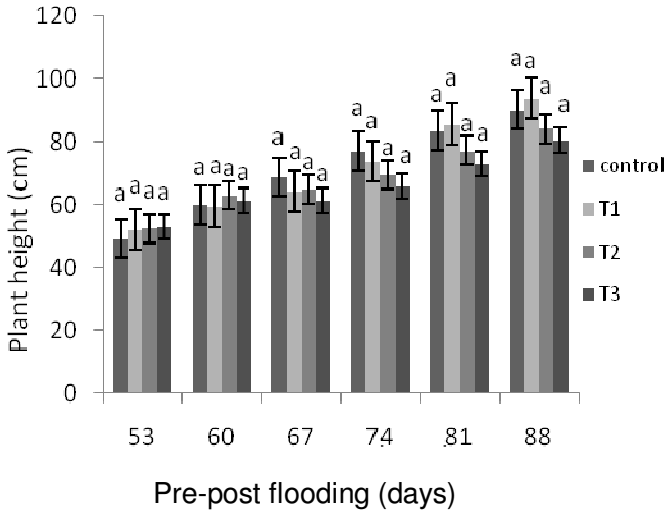

Figure 8. Plant height (cm) of LA1421 grown under flooding conditions.

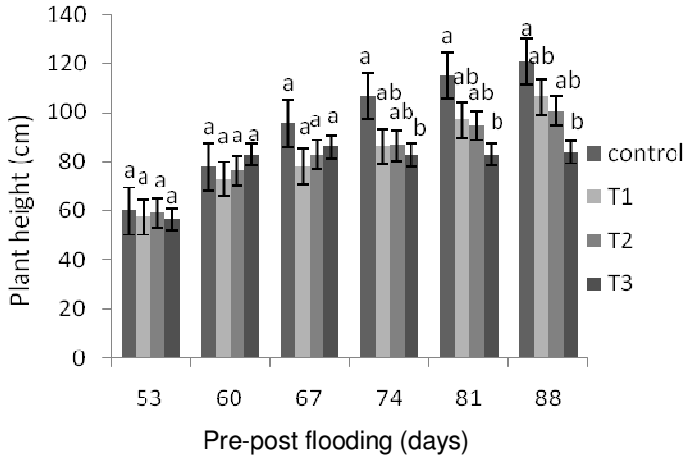

Figure 9. Plant height (cm) of LA1579 grown under flooding conditions.

genotypes were green demonstrating that its chlo-rophyll has not been altered by the deleterious effect of flooding. Advanced senescence of leaves from geno-types LA1421 and LA1579 was recorded (Figures 3 and 4); death of plants were even observed in LA1579 (Figure 4). Leaf epinasty was recorded in CA4.

Flowers and fruits were recorded in CA4 and CLN-2498E (Figures 1 and 2) as genotype CLN2498E bore more fruits than CA4. LA1421 did not bear; neither flowers nor fruits (Figure 3) because as the flowers set in place they dropped immediately. The effect of flooding on LA1579 did not allow the genotype to bear flowers and fruits (Figure 4).

Figure 5 shows that genotypes CA4 and CLN2498E wilted under flooding conditions but recovered from wilting some days later.

ADH and sucrose synthase gene expression

With each flooding treatment, ADH was up- regulated in genotypes CLN2498E and LA1579 compared to CA4 (Figure 10); as the flooding duration increases, ADH gene expression increases especially in LA1579. The relative gene expression in LA1579 was 25, 40, and 100% respectively for 2, 4, and 8 days of continuous flooding. For genotype CLN2498E, 3, 25, and 30 relative gene expression was recorded. There was low gene expression in genotype LA1421.

Figure 11 shows the expression of sucrose synthase; after two days of continuous flooding there were no genotype differences in it, but after 4 days of continuous flooding, sucrose synhtase was down regulated in LA14213 compared to genotypes CA4, CLN2498E and LA1579. After eight days of continuous flooding, CLN2498E sucrose synthase gene expression was up-regulated compared to CA4 whilst LA14213 was down regulated.

DISCUSSION

As a measure for anaerobic metabolism of the leaves, the gene expression of the alcohol dehydrogenase (ADH) and sucrose synthase were determined. In the present study, the high level of gene expression of ADH in LA1579 genotype for 8 days of continuous flooding might have led either to high production and accumulation of ethanol or accumulation of toxic ions. The accumulation of ethanol or toxic ions in root of the plants resulted in the death of LA1579 plants. It has been proposed that the accumulated ethanol may have a “self-poisoning role” in flood intolerant plants. Originally, it was assumed that flood-sensitive species responded to hypoxia with higher ADH activities than tolerant species (Crawford, 1967; McManmon and Crawford, 1971). Several studies supported this relationship (Pezeshki, 1991; Naidoo and Naidoo, 1992; Baruch, 1994; De Simone et al., 2002). However, many authors came to the opposite conclusion (Mendelssohn et al., 1981; Parelle et al., 2006; Keeley, 1979). In general, flood-tolerant species seem to avoid ethanol accumulation in the roots, whereas flood-sensitive species sometimes accumulate this potential cell toxin (Crawford, 1967; McManmon and Crawford, 1971; Monk et al., 1984). Genotypes CA4 and LA1421 produced low ADH meanwhile ADH activity in genotype CLN2498E was reasonable for the genotype to withstand the deleterious effect of flooding even for 8 days of continuous flooding. In a similar study, Johnson et al. (1994) reported that only a small amount of ADH was sufficient for acclimation. Our results is also consistent with that of Benz et al. (2007) who reported that genotypes from flooded habitats did not substantially elevate levels of ADH activity. It is well known that very low O_2 causes accumulations of ethanol and acetaldehyde in fruits and vegetables (Ke et al., 1990).

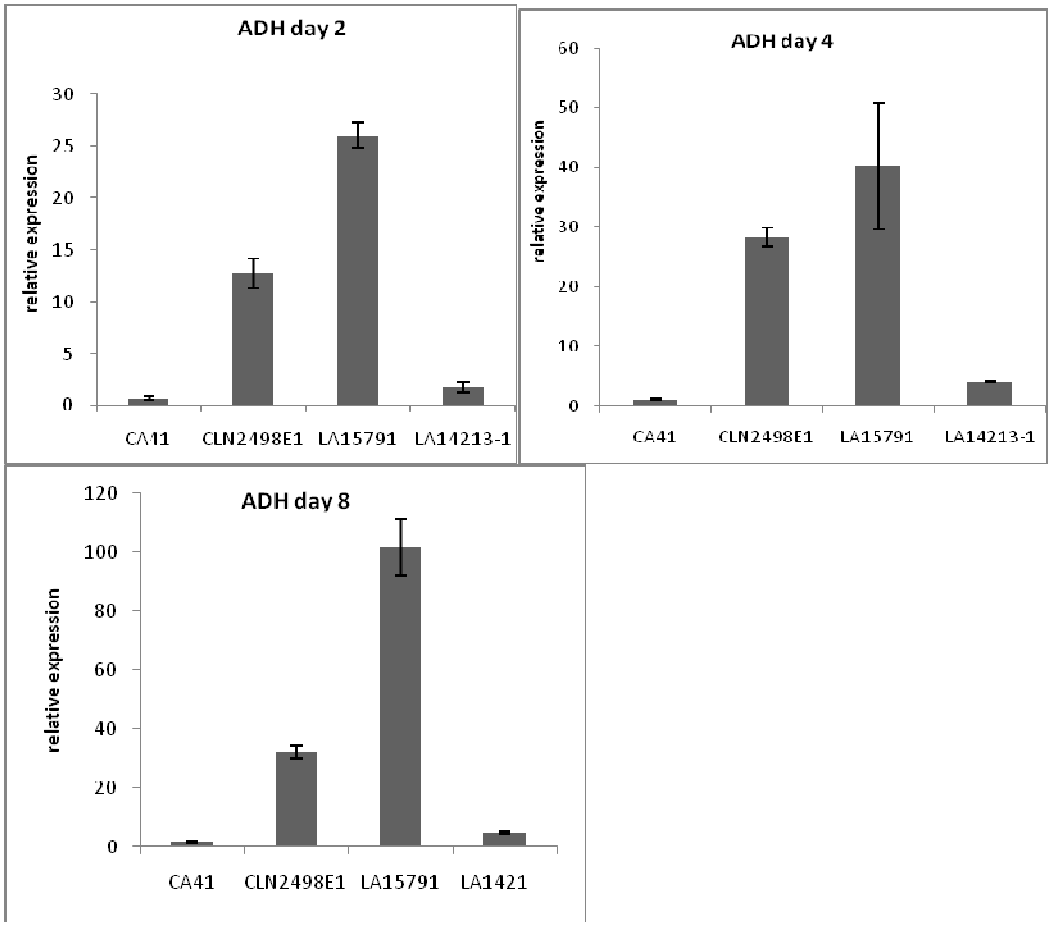

Figure 10. The activity of alcohol dehydrogenase (ADH) for 2, 4, 8 days of continuous flooding (15cm above soil surface)

Plant carbohydrate metabolism is crucially determined by rates of photosynthetic carbon assimilation (source) on the one hand, and carbohydrate consumption in sink tissues on the other hand (Carsten, 2008). Our previous study showed that there was a loss in the yield of PSII photochemistry as a result of reduction of photosynthetic rate (Ezin et al., 2010) in LA1579 genotype, at the same time, there was a very low activity of sucrose synthase in LA1579 genotype among the other genotypes, suggesting decreased amounts of carbohydrates available for respiration, growth, and built-up. This is the reason why all LA1579 plants died after 8 days of continuous flooding. The other three genotypes demonstrated enough activity of sucrose synthase for them to withstand the harmful effect of flooding conditions.

Plants invariably wilt within few hours of 2 - 4 days of imposing a flooding stress (Jackson and Drew, 1984). This is a consequence of higher resistance to mass flow of water through the root. Wilting is caused by the inhibition of respiration and loss of ATP synthesis in the roots. The results of Drew (1984) are consistent with our results where genotypes CA4 and CLN2498 wilted as part of mechanisms put in place to resist flooding conditions. Wilting was also recorded in flooded tobacco (Kramer and Jackson, 1954). Rapid wilting and death of tomato plants after a short of period flooding is usually observed under hot and humid conditions (Drew, 1979). We observed that LA1579 which did not wilt died under 8 days of continuous flooding. This is consistent with the studies of Nunez-Elisea et al. (1999) and Drew (1992), who found that the effect of flooding on plants increased the mortality rates.

Plant height from flooded genotypes CLN2498E and LA1421 did not differ significantly ($P<0.05$) when compared to their control plants whereas in LA1579, there was a different significance in the treatments for 4 and 8 days of continuous flooding. The data obtained indicates that LA1579 genotypes were negatively affected by flooding conditions. The negative effect of flooding in plant growth from genotype LA1579 could be due to reduction of photosynthetic rate (Ezin et al., 2010). Significant difference ($P<0.05$) in CA4 was recorded only for 8 days of continuous flooding. Striker et al. (2007) demonstrated that *Lotus tenuis* has been tolerant to flooding and even showed an important reduction in plant growth. Nunez-Elisea et al. (1999) reported that flooding effects on

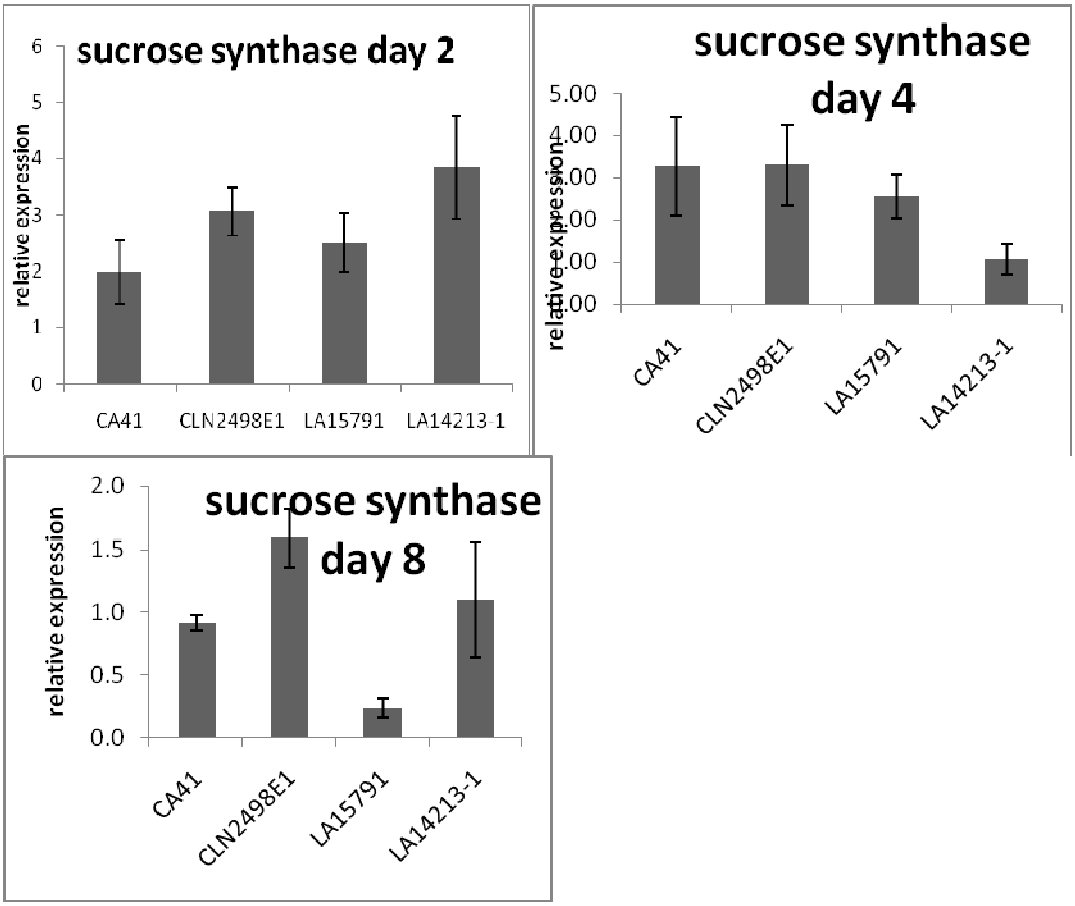

Figure 11. The activity of sucrose synthase for 2, 4, and 8 days of continuous flooding (15cm above soil surface).

plants vary from species to species and reduced growth, other authors finally mentioned that flooding significantly reduces plant height (Andrade et al. 1999; Striker et al., 2008; Yeboah et al., 2008).

No fruits were recorded in LA1579 and LA1421 for 8 days of continuous flooding; this could be due to the fact that this genotype was negatively affected by flooding. Flooding has been a big problem for plants to produce high yield (Lauer, 2008). Dennis et al. (2000) founded that crop losses vary from 10 to 15% to even more than 50% and further stated that waterlogging and flooding can seriously reduce yield.

Conclusion

Generally, plants adjust its morphological, physiological and biochemical functions to respond and adapt to the environmental conditions. In the present study, we conclude that: flood tolerant species are able to synthesize macromolecules such as ADH, and sucrose, and capable of protection against post-flooding injury; genotype LA1579 died for 8 days of continuous flooding; CA4 and CLN2498E produced high yield compared to the two other genotypes; LA1579 was very sensible; LA1421 was moderately tolerant, CA4 and CLN2498E were tolerant to flooding.

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