Full Length Research Paper

Effect of salt stress on flowering, fructification and fruit nutrients concentration in a local cultivar of chili pepper (Capsicum frutescens L.)

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Received 27 March, 2019; Accepted 13 May, 2019

Salt stress is one of the major environmental constraints limiting agricultural productivity and influencing the concentration of bioactive compounds of vegetables. In this study, we assessed the effect of NaCl salt stress on flowering, fructification and fruit nutritional quality of a local cultivar of chili pepper. The experiment was carried out in a screen house as a completely randomized design (CRD) with three replications. Three weeks old plants were submitted in pots containing a mixture of potting soil and sand, to five NaCl concentrations; 0, 30, 60, 90 and 120 mM NaCl by irrigation every two days for 94 days. Salinity retarded significantly flowering and fruit ripening, and reduced significantly the fruit number, as the NaCl concentration increased with no fruit obtained at 90 and 120 mM NaCl. Fruit size and fresh mass were also significantly reduced by salt stress. Capsaicinoids contents increased significantly for about 389% in comparison to the control at 60 mM NaCl, whereas vitamins B6, B12 and C contents decreased significantly with increasing NaCl concentration. Thus, salt stress retarded flowering and fruit ripening; reduced fruit number, size and mass; enhanced fruit tangy appearance and deteriorated fruit nutritional values in chili pepper.

Key words: chili pepper, local cultivar, NaCl, capsaicinoids contents, vitamins contents, fruits growth, Benin.

INTRODUCTION

Chili (Capsicum spp.) is a spice, a fruit vegetable widely grown in the world as it is very important in human food (Dias et al., 2013; Wahyuni et al., 2013). It is one of the three important solanaceous vegetable crops grown for their fruits, which are consumed, either fresh or dried (Hedge, 1997). Chili pepper belongs to the crops grown...
Table 1. Chemical composition of the experimental soil.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic matter (%)</td>
<td>0.79</td>
</tr>
<tr>
<td>Organic carbon (%)</td>
<td>0.58</td>
</tr>
<tr>
<td>Total nitrogen (%)</td>
<td>0.05</td>
</tr>
<tr>
<td>C/N</td>
<td>8.14</td>
</tr>
<tr>
<td>Assimilable phosphorus (ppm)</td>
<td>64.25</td>
</tr>
<tr>
<td>Potassium (K⁺) (meg/100 mg)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

throughout the world for their nutraceutical (nutritional and medicinal) and economic virtue (Rahman et al., 2013). In Benin, chili is the second cash gardening crop besides tomato (Assogba, 2009). Its annual production is about 47,162 t and has never evolved from 2000 to 2009 (Assogba, 2009). Pepper plants produce the phenolic compound capsaicin, primarily in the placenta of fruits, possibly to deter mammalian herbivores in favor of avian frugivores (Arrowsmith et al., 2012). According to these authors, capsaicin content is influenced by genetic and environmental conditions including soil moisture and fertility, temperature and light. Moreover, except researches on natural foes conducted in different production zones and the few agronomic assessment tests conducted on certain varieties (Assogba, 2009), no other significant study has been conducted on chili in Benin. It is classified as moderately sensitive to salinity (Maas and Hoffman, 1977), and some adverse effects of salinity on this species have been reported (De Pascale et al., 2003; Navarro et al., 2003; Villa-Castorena et al., 2003; Huez-López et al., 2011). In Benin, chili pepper is grown only for food (Orobiyi et al., 2017) partially in the cultivable lands of the coastal areas, where soil salinity and water irrigation are a reality. Salt stress is one of the major environmental constraints limiting agricultural productivity (Wei et al., 2003). This stress is known to negatively affect plant growth at all developmental stages, but sensitivity varies greatly at different stages (Akram et al., 2002; Akinci et al., 2004). Crop production in saline areas largely depends on successful germination, seedling emergence and establishment and efficient reproductive phase (Akinci et al., 2004). Moreover, as environmental stress, it may have a strong influence on the concentration of bioactive compounds of vegetables (Prasad et al., 2014).

However, despite a substantial amount of literature on responses of plants to salinity stress, data on the effect of salt stress on flowering, fructification and on nutrient contents of fruits are lacking. Moreover, only few research works have focused on the response of chili cultivars produced in Benin to salt stress. In a recent study, we demonstrated that salt stress reduced plant growth in five chili cultivars produced in Benin, and that there is a variability in salt tolerance of these cultivars (Kpínkoun et al., 2019). Since chili pepper is mainly used in Benin as fruits vegetable, it is important to know if NaCl stress induces a reduction of the fruits number per plant, fruit size and mass and if it induces a modification in fruit nutrient contents. The present study aims to evaluate NaCl stress effects on flowering, fruits number, fruit size, fruit fresh mass, capsaicinoids and vitamins concentrations of one of the main local chili cultivars grown in Benin.

MATERIALS AND METHODS

Plant material

The local cultivar produced in Benin named Adologbo from the species Capsicum frutescens was used. Seeds were obtained from the market gardening crops program of the Benin National Institute for Agricultural Research (INRAB).

Experimental conditions

The experiment was carried out in a screen house at Center for Agricultural Research of Agonkanmey (Abomey-Calavi, Benin Republic) from February to May 2017. Plants were cultivated at a temperature of 26°C/22°C day/night with natural light and a relative humidity of 55%. Seeds were incubated for germination in tanks filled with potting moistened soil for two weeks. Young seedlings were then transferred to earthen small pots of 5.8 cm diameter and 6 cm height containing a mixture of potting soil and sandy loam soil 50:50 (one plant/pot) and cultivated for one week before stress application. The chemical composition of the soil used in organic matter, nitrogen, organic carbon, phosphorus and potassium was determined at the soil science, water and environment laboratory of the Center for Agricultural Research of Agonkanmey (Abomey-Calavi, Benin Republic) and described in Table 1. Plants of cultivar Adologbo of 21 days old were submitted to salt stress in earthen big pots of 11.3 cm diameter and 14 cm height filled with 3 kg of a same mixture for 94 days. Treatments consisted in plant irrigation every two days with 100 ml/pot of 0, 30, 60, 90 or 120 mM NaCl solution corresponding respectively to an electric conductivity of 0.221, 3.827, 6.47, 10.56 and 14.02 dS.m⁻¹ determined by a conductometer (VWR; CO310). The experiment was laid out as a completely randomized design (CRD) with one factor (NaCl concentrations) and three replications.

Flowering evaluation

The date of appearance of the first flowers was noted for each treatment; thus, the number of days set by each plant to give its first flower from the start of the stress application was raised.

Fruit ripening, number, size and fresh mass determination

The date of appearance of the first ripe fruits (having reached the physiological maturity determined by the first signs of color change) was recorded for each treatment. Thus, the number of days set by each plant to give its first ripe fruits from the start of stress application was raised. Ripe fruits were collected and counted for each treatment from their appearance to 45 days after the first fruit ripening (approximately 94 days from the start of stress application). The first ripe fruits samples from each treatment were photographed and two ripe fruits were selected from each plant and weighed. Thus, a total of six (6) fruits were weighed per treatment to determine the average fresh mass of each fruit for each treatment. The average fresh mass of each pepper fruit is obtained for each treatment as the means of the fresh mass of the six (6) fruits.
Table 2. Effect of different NaCl concentrations on flowering, fruits ripening and fruits number of chili pepper cv. Adologbo: Values are means±SEs.

<table>
<thead>
<tr>
<th>NaCl (mM)</th>
<th>Date of flowering (days)</th>
<th>Date of first fruit ripening (days)</th>
<th>Number of ripe fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>23.66±1.76&lt;sup&gt;c&lt;/sup&gt;</td>
<td>48±1.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.33±0.88&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>23.66±1.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>52.66±1.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.66±0.66&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>60</td>
<td>31.66±3.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66.66±1.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.33±0.33&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>90</td>
<td>39.66±3.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>0±0.00&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>120</td>
<td>40.33±1.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>0±0.00&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means with different letters within a column were significantly different (p≤0.05).

Figure 1. Effect of different NaCl concentrations (0, 30 and 60 mM) on fruits size of chili pepper cv. Adologbo.

**Fruit nutrients determination**

Nutrient contents were determined in mature fruits collected 15 days after the ripening of the first fruit. The mature fruits were used for estimating capsaicinoids (capsaicin and dihydrocapsaicin), vitamin B6 (pyridoxine), vitamin B12 (cobalamine) and vitamin C (ascorbic acid) contents. Capsaicinoids were determined using the method of Al Othman et al. (2011) with few modifications; vitamins B6 and B12 with the method of Sami et al. (2014) with few modifications; vitamin C with the method of Karboue and Nesrallah (2014).

**Statistical analysis**

Each value was presented in the form of mean ± standard error. For number of days and number of fruits, Kruskal-Wallis test was used to compare the effect of stress intensity with Minitab software (Minitab, 2010). For all other parameters, analysis of the main effects of stress intensity was based on a one-way analysis of variance (ANOVA) and differences among means were compared through Student, Newman and Keuls (SNK) test using JMP Pro software (JMP Pro SAS Institute, 2009).

**RESULTS**

**NaCl effect on flowering, fruits ripening and fruits number**

NaCl effect on the date of appearance of the first flowers is shown in Table 2. The date increased from 23.66 days for the control to 31.66, 39.66 and 40.33 respectively at 60, 90 and 120 mM NaCl corresponding to a delay of 8, 16 and 17 days of the flowering in comparison to the control; however, no change was observed at 30 mM NaCl. Thus, NaCl effect on chili plant resulted in a delay of the flowering.

NaCl effect on fruits ripening is shown in Table 2. No fruit was obtained at 90 and 120 mM NaCl. An increase was observed in the time of the appearance of the first ripe fruit under salt stress causing a delay of 4 and 18 days respectively for 30 and 60 mM NaCl in comparison with the control. This increase was significant only at 60 mM NaCl; thus, NaCl effect on chili fruit resulted in a significant delay of fruit ripening. NaCl effect on ripe fruits number is shown in Table 2. A number of ripe fruits decreased from 6.33 in the control to 3.66 and 2.33 respectively at 30 and 60 mM NaCl. The fruit fresh number reduction under salt-stress corresponded to 42 and 63% in comparison with the control respectively at 30 and 60 mM NaCl. Thus, NaCl effect on chili fruit resulted in a significant decrease in ripe fruit number.

**NaCl effect on fruit size and fresh mass**

NaCl effect on fruits size is shown in Figure 1. A decrease in fruit size was observed at all NaCl concentrations used; thus, NaCl effect on chili fruit resulted in a decrease in fruit size. NaCl effect on fruits fresh mass is shown in Figure 2. A significant decrease is observed at all NaCl concentrations used. Fruit fresh mass decreased from 5.7 g in the control to 2.08 and 0.74 g respectively at
Figure 2. Effect of different NaCl concentrations on fruits fresh mass (mg) of chili pepper cv. Adologbo: Values are means of 6 fruits (2 per replication). Vertical bars are standard errors. Means with different letters were significantly different (p< 0.05).

Table 3. Effect of different NaCl concentrations on fruits capsaicinoids and vitamins contents (µg g⁻¹ fm) of chili pepper cv. Adologbo: Values are means ± SEs, n = 3.

<table>
<thead>
<tr>
<th>NaCl (mM)</th>
<th>Capsaicinoids</th>
<th>Vitamin B6</th>
<th>Vitamin B12</th>
<th>Vitamin C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>60.5±3.67ᵃ</td>
<td>144412±15357ᵃ</td>
<td>5452.13±385.42ᵇ</td>
<td>435.47±44.8ᵃ</td>
</tr>
<tr>
<td>30</td>
<td>90.81±5.08ᵇ</td>
<td>91387±7822ᵇ</td>
<td>2655.84±455.2ᵇ</td>
<td>319.95±16.5ᵇ</td>
</tr>
<tr>
<td>60</td>
<td>295.77±19.5ᵇ</td>
<td>34525±12405ᵇ</td>
<td>966.41±264.8ᵇ</td>
<td>151.82±63.4ᵇ</td>
</tr>
</tbody>
</table>

Means with different letters within a column were significantly different (p< 0.05).

30 and 60 mM NaCl. The fruit fresh mass reduction under salt-stress corresponded to 64 and 87% in comparison with the control respectively at 30 and 60 mM NaCl. Thus, NaCl effect on chili fruit resulted in a significant decrease in individual fruit fresh mass.

**NaCl effect on capsaicinoids and vitamins concentrations**

NaCl effect on fruits capsaicinoids and vitamins concentrations is shown in Table 3. As no fruit was obtained for plants cultivated in the presence of 90 and 120 mM NaCl, only fruits from 0, 30 and 60 mM NaCl were used for nutrients determination. A significant increase (p<0.05) was observed for capsaicinoids. These increases were 50 and 389% in comparison with the control respectively at 30 and 60 mM NaCl. Salt effect resulted in a significant decrease for vitamin B6 (p<0.01), B12 (p<0.001) and C (p<0.05). The decreases were 76, 82 and 65% in comparison with the control respectively for vitamins B6, B12 and C at 60 mM NaCl. Thus, NaCl effect on chili fruit vitamins contents resulted in a significant decrease for vitamins B6, B12 and C.

**DISCUSSION**

Plant growth is compromised by salinity at all developmental stages, but sensitivity varies greatly at different stages (Gandonou and Skali, 2015). Crop production in saline areas largely depends upon successful germination, seedling emergence and establishment and efficient reproductive phase (Akinci et al., 2004). Salt stress leads to suppression of plant growth and development at all growth stages, however, depending upon plant species, certain stages such as germination, seedling or flowering stage could be the most critical stages for salts stress (Khoshosokan et al., 2012). In the Benin costal vegetable crops management system, plants were irrigated from young plants stage to harvest two times every day. It is important for studying salt effect on reproductive stage in such context, to irrigate plants from young plants stage to flowering and fructification. Our results revealed that increasing salinity
applied from the young plant stage delayed plant flowering and fruit ripening. In Iris hexagona, Van Zandt and Mopper (2002) reported that salinity strongly delayed flowering phenology mainly in the second year when less than 4 g/L NaCl delayed flowering up to 3 days. For these authors, as Iris flowers are receptive to pollinators for 2 days or less, this 3-day delay could affect outcrossing dynamics, and ultimately, the evolutionary ecology of iris populations. In general, the experiment related to salt stress effect on plant reproductive stage started later when plants are ready to flower. Generally, those studies did not reveal a delay of flowering. A long-term exposure is necessary to this kind of effect as reported in I. hexagona where a one-year experiment would have failed to reveal the strong effects of salinity that emerged in the second year. However, in the ornamental annual plants Tagetes patula and Ageratum mexicanum, Zapryanova and Atanassova (2014) reported that plants treated with NaCl have earlier and shorter blooming period than non-treated plants indicating that salinity stress accelerate flowering in these species. This opposite reaction can be explained by a genotypic response.

According to Shrivastava and Kumar (2015), salinity adversely affects reproductive development by inhabiting microsporogenesis and stamen filament elongation, enhancing programed cell death in some tissue types, ovule abortion and senescence of fertilized embryos. These effects were the results of a low osmotic potential of soil solution (osmotic stress), specific ion effects (salt stress), nutritional imbalances, or a combination of these factors (Ashraf, 2004). In our study, salt stress also decreased fruit number, size and fresh mass in our chili pepper cultivar. Similar results were reported in other chili pepper cultivar Sandia by Huez-Lopez et al. (2011) who observed that the mean fresh fruit yields decreased as soil salinity increased. In three other chili pepper cultivars, R’him et al. (2013) reported that salinity reduced the percentage of fruit set, yield and average fruit weight corroborating our results. However, Huez-Lopez et al. (2011) found that, in chili pepper cultivar Sandia, fruit number was more affected by salinity than the individual fruit weight. Apparently, it is not the case in our local cultivar as the individual fruit fresh mass reduction under salt-stress was higher (64 and 87% respectively at 30 and 60 mM NaCl) than fruit number reduction (42 and 63% respectively at the same NaCl concentrations). Thus, individual fruit fresh mass was more affected than fruit number in cultivar Adologbo. These opposite results can be explained by a genotype dependent response. In tomato, Parvin et al. (2015) reported similar tendency indicating that the fruit weight is gradually decreased with the increased levels of salinity. In vegetable crops, it is well known that salt stress decreases marketable yield due to decreased productivity and an increased unmarketable yield of fruits, roots, tubers and leaves without commercial value (Machado and Serralheiro, 2017). It is the case in sweet pepper cultivar Somontano if we referred to Rubio et al. (2009), and for our chili pepper cultivar Adologbo. According to R’him et al. (2013), the marketable fruit reduction by salt treatment was mainly due to the increase in the number of fruit affected by blossom-end rott.

Vegetables contain nutritional values provided by sugars, proteins, lipids, minerals, antioxidants, vitamins, etc. (Prasad et al., 2014). According to these authors, increasing environmental stresses has strong influence on the concentration of bioactive compounds while affecting the valuable constituents of vegetables which are getting deteriorated day by day. Chili species are known to be a rich capsaicinoids source. Our results revealed an increase in capsaicinoids concentrations of fruits. In other cultivar of chili pepper, Arrowsmith et al. (2012) found that fruits of plants cultivated at the two highest salinities (1.0 and 1.5%) had higher levels of capsaicin compared to the control and lowest salinity group but no significant differences were found among NaCl concentrations. These results corroborate our findings and seem to indicate that salt stress effect resulted in an increase in capsaicinoids concentration in chili pepper fruit. In other chili pepper cultivars submitted to water deficit treatment, Sung et al. (2005) reported a higher concentration of capsaicin in fruits of stressed plants than that of control plants in all cultivars tested. However, these authors revealed variability among cultivars when capsaicin concentration was compared between the fruit placenta and pericarp. No significant different was observed in the concentration of capsaicin in the placenta of cultivar Hungariana, whereas it reached 3.84-fold that of the control for cultivar Beauty Zest. These authors also revealed that the activity of the main enzyme implicated in the synthesis of capsaicinoids (capsaicinoid synthetase) was 1.45 to 1.58-fold higher in fruits in the water deficit treatment than in fruits in the control treatment 40 days after flowering. It is therefore logical to suppose that the increase in capsaicinoids concentration of fruits from NaCl-stressed plants observed in our study should be attributed, at least partly, to the increase in the activity of capsaicinoid synthetase.

Our results revealed that pyridoxine (vitamin B6) and cobalamin (vitamin B12) contents in the fruit decreased with salt concentration. Studies of environmental stresses effects on B vitamins in plants are very scarce. Pyridoxine (vitamine B6) is a cofactor for many enzymatic reactions, especially those involved in amino acid metabolism; it is thus required by all organisms (Trotel-Aziz et al., 2003). This vitamin is required for plant development and tolerance to oxidative radicals generated by abiotic stresses (Chen and Xiong, 2005). According to Hanson et al. (2016), B vitamins are prone to destruction under stress conditions. Our results seem to confirm this idea and let us to suppose that stressed plants suffer B vitamin deficiencies and that certain stress symptoms are metabolic knock-on effects of these
deficiencies, mainly those related to oxidative radicals scavenging. Ascorbic acid (vitamin C) is an essential nutrient which occurs widely in crop foods products, especially in fresh fruits and green leafy vegetables (Ratnakar and Rai, 2013). It is a small, water soluble, antioxidant molecule which acts as a primary substrate in the cyclic pathway of enzymatic detoxification of hydrogen peroxide (Beltagi, 2008). Vitamin C also helps in absorption of dietary iron by keeping it in the reduced form (Ratnakar and Rai, 2013). Our results reveal that in fruits of C. frutescens cv. Adologbo, the ascorbic acid content decreased significantly under NaCl stress. In other vegetables such as amaranth species leaves, Ratnakar and Rai (2013) observed a decrease of ascorbic acid content with increase of salt concentration, whereas Wouyou et al. (2017) reported an opposite response in other amaranth cultivar. In tomato fruits, the increase of ascorbic acid contents under salt stress was reported (Stamatakis et al., 2003; Kim et al., 2008; Gautier et al., 2010). The results of the present study reveal that salt stress decreased chili pepper fruit nutritional quality by mainly decreasing vitamins concentrations but increase fruit tangy appearance by increasing capsaicinoids concentration.

Conclusion
This study indicated that increasing NaCl concentrations delayed significantly flowering and fruit ripening and reduced significantly fruits number, size, fresh mass and vitamins B6, B12 and C concentrations, but increased capsaicinoids concentration and consequently fruit tangy appearance. Thus, salt stress reduced the fruit yields and deteriorated fruit nutritional quality by reducing vitamins concentrations. Further study is necessary to check the implication of capsaicinoid synthetase activity in the increase of the capsaicinoids concentration under salt stress in our local chili cultivar fruits.

CONFLICT OF INTERESTS
The author has not declared any conflict of interests.

ACKNOWLEDGEMENTS
The author thanks Professor Alexandre Dansi Anagonou from University of Abomey-Calavi and Mr Patrice Amoussou for the reading and corrections made to the manuscript.

REFERENCES


