Physiological responses of *Amaranthus hybridus* L. under salinity stress

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Abstract

Soil salinity is an abiotic factor that adversely affects growth and development of plants. To evaluate the physiological responses of *Amaranthus hybridus* L. under salinity stress, seedlings were exposed to 0, 0.1 and 0.2M NaCl for a period of 6 weeks. Whole plant dry weight, relative water content (RWC), total chlorophyll, lipid peroxidation, protein level and antioxidant enzymes activities were evaluated after the treatment period. The results of the study showed that salinity caused a significant decrease in whole plant dry weight, relative water content, total chlorophyll and protein content while an increase in malondialdehyde content, catalase and ascorbate peroxidase activities were observed. The severity of these effects was concentration dependent. The biomass accumulation of the control plants was 11.67±0.39 g, while those that received 0.1 and 0.2 M NaCl had 9.22±0.28 and 6.94±0.07 g respectively. The increase in malondialdehyde content and antioxidant enzymes activities were indications that salinity stress induced the production of reactive oxygen species (ROS) which caused oxidative damage to macromolecules in living cells.

Keywords: Salinity; absorption; lipid peroxidation; oxidative stress; *Amaranthus hybridus*

Abbreviations: TCA- Trichloroacetic acid; DW- Dry weight; SW- Saturated weight; RWC- Relative water content; H_2O_2 - hydrogen peroxide; ROS - Reactive oxygen species; MDA- Malondialdehyde; CAT - Catalase; APX - Ascorbate peroxidase; SE - Standard error; ANOVA - One-way analysis of variance.

1. Introduction

Agricultural productivity worldwide is subjected to increasing environmental constraints, particularly to salinity due to its high magnitude of impact and wide distribution. It leads to a deterioration of land and reduction in crop productivity worldwide (Chinnusamy *et al*., 2005). Large areas of arable lands are being lost every year due to increasing soil salinity and therefore not suitable for crop production. Excessive amounts of salt in the soil, most commonly NaCl, have detrimental effects on the plant growth and productivity (Zilli *et al*., 2008; Sobhanian *et al*., 2010). Soil salinity stresses plants in two ways: high concentrations of salts in the soil make it difficult for roots to absorb water; and high concentrations of salts within the plant can be toxic (Munns & Tester, 2008). Most of this salt affected land has arisen from natural causes, from the accumulation of salts over long periods of time in arid and semiarid zones (Rengasamy, 2002). Weathering of parental rocks releases soluble salts of various types, mainly chlorides of sodium, calcium, and magnesium, and to a lesser extent, sulphates and carbonates.

Plants exposed to stresses undergo changes in their metabolism in order to adapt with changes in their environment. Salinity changes the morphological, physiological and biochemical responses of plants. Under salinity stress, the osmotic potential in the soil solution exceeds the osmotic potential of plant cells due to presence of higher concentration of salt which reduces the ability of plants to take up water and other essential nutrients (Munns *et al*., 2006). The plants are exposed to both osmotic stress and specific ion toxicities during the salinity stress. Salinity stress has both osmotic (cell hydration) and toxic (ion accumulation) effects on plants (Joseph & Jini, 2011). According to Carvajal *et al*. (1999); Yeo (1998); Grattan & Grieve (1999), the direct effects of salts on the plant growth may be divided into three broad categories: (i) a reduction in the osmotic potential of the soil solution that reduces plant available water, (ii) a deterioration in the physical structure of the soil such that permeability and soil aeration are diminished, and (iii) increase in the concentration of certain ions that have an inhibitory effect on the plant metabolism(specific ion toxicity and mineral nutrient deficiencies). Osmotic effects of salts on plants are a result of lowering the soil water potential due to increasing solute concentrations in the root zone.

At low soil water potentials, this condition interferes with the plant's ability to absorb water from the soil and maintain turgor. However, at low or moderate salt concentrations (high soil water potentials), plants adjust osmotically and maintain a potential for the influx of water (Ghoulam *et al*., 2002). At high salinity, some specific symptoms of plant damage may be recognized such as necrosis and leaf tip burn due to Na^+ or Cl^- ions (Wahome *et al*., 2001). High ionic concentrations may disturb membrane integrity and function; interfere with internal solute balance and nutrient uptake, causing nutritional deficiency symptoms similar to those that occur in the absence of salinity (Grattan & Grieve, 1999).

Sodium and chloride, usually the most prevalent ions in saline soils or water, account for most of the deleterious effects that can be related to specific ion toxicities. The degree to which growth is reduced by salinity differs greatly with species and to a lesser extent with varieties (Ghoulam *et al*., 2002). High salt (NaCl) uptake competes with the uptake of other nutrients ions, such as K^+, Ca^{2+}, N, P resulting in nutritional disorders and eventually, reduced yield and quality (Grattan & Grieve, 1999). Entry of sodium and chloride ions in large amount in chloroplasts leads to several disorders. According to Munns & Tester (2008), induced nutritional disorders are one of the noticeable effects of salinity stress in plants. The normal absorption of mineral nutrition is affected by salinity because the distribution of essential nutrients is mainly influenced by NaCl.

Plant salt resistance has been defined by Shannon & Grieve (1999) as an inherent ability of plants to withstand the effects of high concentrations in the root zone or on the leaves without a significant adverse effect.

Appropriate management options are required to prevent or alleviate salinity problems in crop production. Among several strategies devised to overcome the problem of salinity stress, the selection of crop species or cultivars with salinity tolerance traits has been considered an economical and efficient strategy. Adequate understanding of responses of plants to salinity stress is required for accurate selections to be achieved and may also open a way to crop manipulation in order to improve on their tolerance. There is paucity of data in Nigeria concerning the effects of salinity stress on locally cultivated leafy vegetables, as most past studies have being on the grass family. Therefore, this study was carried out to evaluate the responses of *Amaranthus hybridus* to simulated salinity stress in order to contribute to the existing data concerning this subject in Nigeria.

2. Materials and methods

2.1 Plant growth and treatment

Seedlings were raised on a nursery bed for 2 weeks. Healthy looking seedlings of 12 cm in height were transplanted into polythene bags filled with sandy loam soil to achieve a plant stand of 1 seedling per bag, and were arranged in a randomized block design in a screen house. They were watered every 2 days and allowed to acclimatize for 2 weeks before they were subjected to salinity stress. Seedlings were treated with 200 ml 0.1 and 0.2 M NaCl every 2 days for 6 weeks. The control plants received equal volume of water for the same period.

2.2 Biomass determination

Harvested plants were washed thoroughly in running tap water and rinsed twice with distilled water. They were then placed in paper bags and weighed after oven-dried at 80 ^oC for 72 h.

2.3 Relative water content (RWC)

The fourth leaf of the plants representing each treatment were harvested and weighed to determine their fresh weight (FW). The leaves were submerged separately in distilled water for 24 h in the dark. They were removed from the water after this period, mopped dry using an absorbent and weighed to determine their saturated weight (SW). The leaves were then placed in envelopes and dried in an oven at 80 ^oC for 24 h, the weights were then taken to get the dry weight (DW). The relative water content was calculated according to Turner (1981) using the formula: RWC (%) = [(fresh weight - dry weight)/(saturated weight - dry weight)] x 100.

2.4 Determination of total chlorophyll

Plant leaves (0.5g) were ground in 10ml 80% acetone in the dark. After centrifugation at 4000 g for 5 min, the absorbance of the supernatant was read at 645 and 663 nm (Arnon, 1949). The total chlorophyll content was calculated using the formula given by Machlachlan & Zalik (1963).

2.5 Lipid peroxidation

Lipid peroxidation was measured by estimation of the malondialdehyde (MDA) content following a modified procedure of Wang & Jin (2005). Fresh leaves (0.5 g) were homogenized in 5 ml 20% trichloroacetic acid (TCA). The homogenate was centrifuged at 10000g for 5 min. The supernatant (1ml) was mixed with equal volume of 0.6% (w/v) thiobarbituric acid solution comprising 10% TCA. The mixture was incubated for 30 min in a boiling water bath and cooled quickly on ice bath. The absorbance of the mixture was read at 450, 532 and 600 nm. The concentration of MDA was calculated as 6.45 $(A_{532}-A_{600})-0.56\ A_{450}$.

2.6 Enzyme determination

For enzyme analysis, fresh samples of leaves (100 mg each) were ground in a ceramic mortar and extracted with 5 ml of 100 mM potassium phosphate buffer pH 7.5, with 1% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 12,000 rpm for 5 min. The supernatant was used for the estimation of antioxidant enzyme activities. Catalase (CAT) activity was determined according to Aebi (1984), by monitoring the decrease in the absorbance at 240nm as a consequence of H_2O_2 disappearance. Ascorbate peroxi-

dase (APX) activity was assayed according to the method of (Nakano & Asada, 1981). The oxidation of ascorbate was determined by the change in absorbance at 290nm.

2.7 Protein determination

The protein level was determined using the micro-Kjeldahl method for the estimation of total organic nitrogen in the dried plant samples as described by Piorreck *et al*., (1984). The total nitrogen content was multiplied by 6.25 to obtain the amount of protein.

2.8 Measurement of tissue Na and K ions

Na^+ and K^+ were measured according to the method described by Wang *et al*. (2007). Na^+ and K^+ were extracted from the dried plant tissues in 100 mM acetic acid at 90 ^oC for 2 h, and ion analysis was performed using an atomic absorption spectrophotometer (Pye Unicam 2000).

2.9 Statistical analysis

Means of three replicates as well as their standard errors (SE) were determined. The test of significance between the treatments was done using a one way analysis of variance (ANOVA).

3. Results

The effect of salinity stress on the dry weight of *Amaranthus hybridus* seedlings is depicted in Figure. 1. The result showed that salinity significantly decreased whole plant dry weight and the effect increased with salt concentration. The control plants had a mean dry weight of 11.67 ± 0.39 g. This value was significantly higher than the mean values observed in plants that received 0.1 M and 0.2 M NaCl which had 9.22 ± 0.28 and 6.94 ± 0.07 grams respectively.

Figure. 1 *Whole plant dry weight of Amaranthus hybridus under salinity stress*

Figure. 2 *Relative water content of Amaranthus hybridus under salinity stress.*

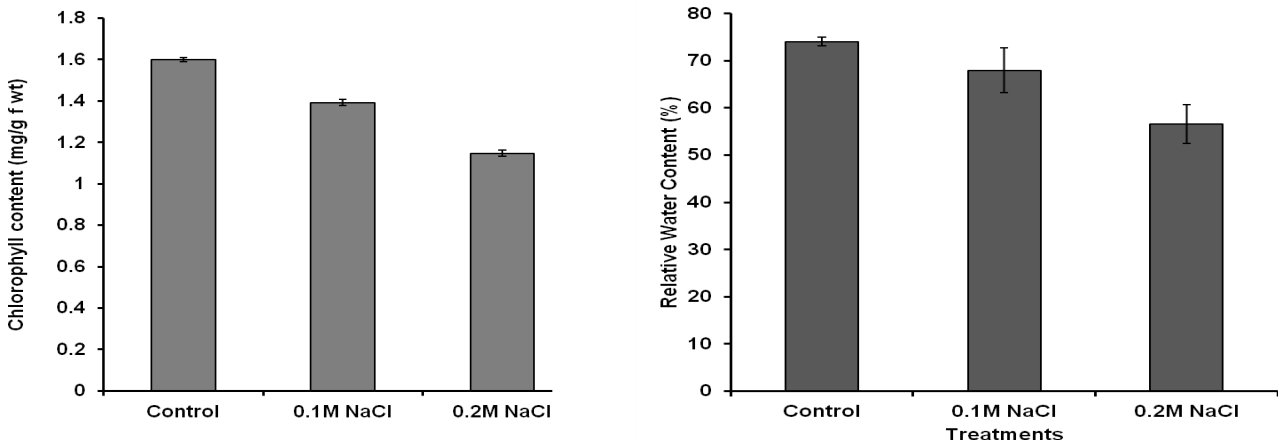

Figure 2 represents the mean Relative Water Content of the plants at different levels of NaCl treatment. Relative water content (RWC) in the leaves of *Amaranthus hybridus* was affected by salinity. The control plants had RWC value of 74%, this was significantly higher than the respective values observed for 0.1 and 0.2M NaCl treated plants which had 68% and 57%. It was observed in this study that salinity stress caused a significant reduction in total chlorophyll in the leaves. Leaf chlorophyll was significantly reduced when plants were exposed to prolonged salinity treatment (Figure 3).

To evaluate lipid peroxidation under salinity stress, Malondialdehyde (MDA) content was determined. Salinity led to a gradual increase in MDA levels in the plants. MDA accumulation was highest in plants subjected to 0.2M NaCl with a mean value of 1.633 mg/g. Plants treated with 0.1M NaCl had a mean value of 0.806 mg/g while the control plants had 0.436 mg/g (Figure 4).
The activities of catalase (CAT) and ascorbate peroxidase (APX) were evaluated as representative enzymes involved in antioxidant metabolism. The activities of the two antioxidant enzymes followed the same trend when *A. hybridus* seedlings were exposed to salinity stress. It was observed that salinity treatment generally led to a significant increase in the activities of these enzymes, indicating salinity- induced oxidative stress. Plants treated with 0.2M NaCl had the highest catalase activity of 38.12 ± 0.41 µmol/min/g f wt, while the control plants had the least mean value of 18.36 ± 0.28 µmol/min/g f wt. It was observed that the responses caused by salinity stress were concentration dependent (Fig. 5). In the same vein, plants exposed to 0.2M NaCl had the highest APX activity

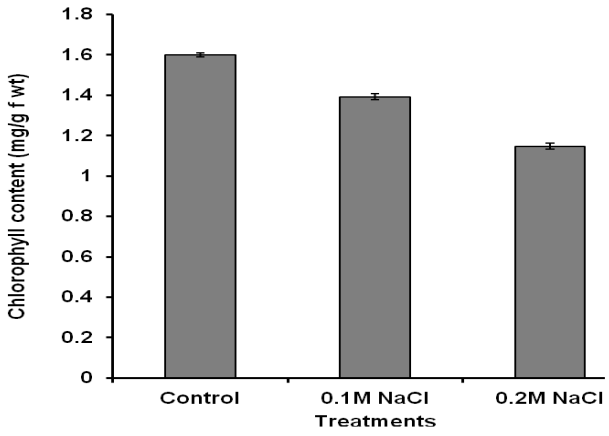

Figure. 3 *Chlorophyll content of Amaranthus hybridus subjected to salinity stress.*

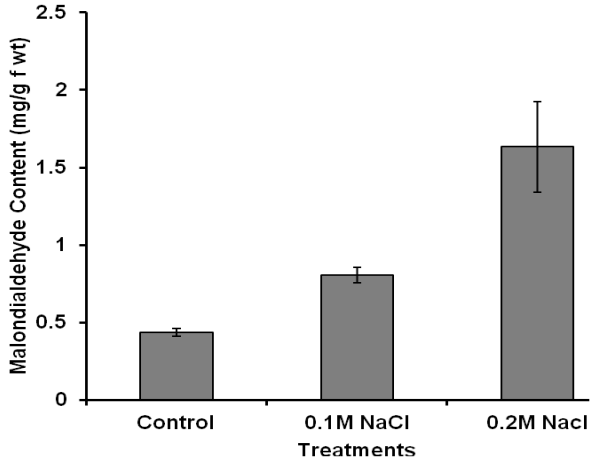

Figure.4 *Malondialdehyde content of Amaranthus hybridus exposed to different concentrations of sodium chloride solution.*

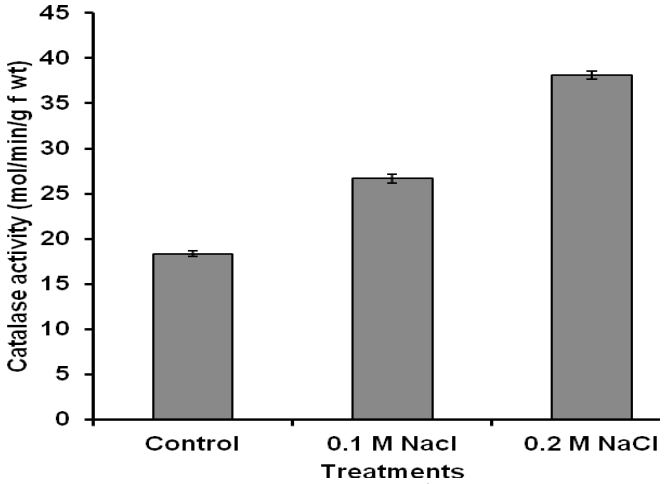

Figure. 5 *Catalase activity in the leaves of Amaranthus hybridus under salinity stress.*

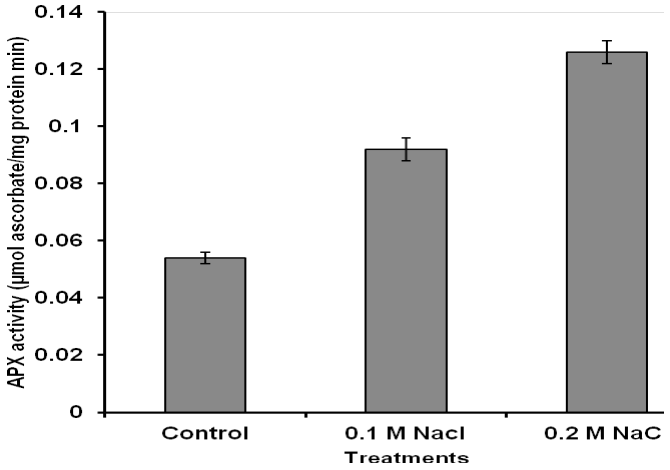

Figure.6 *Ascorbate peroxidase activity in the leave of Amaranthus hybridus under salinity stress.*

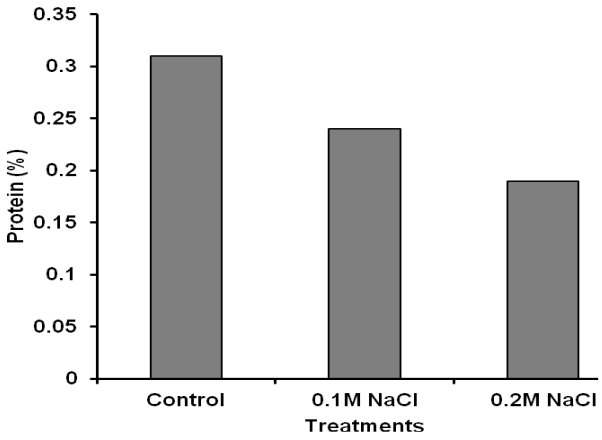

Figure.7 *Percentage protein content of Amaranthus hybridus (g-1d. wt) exposed to different concentrations of salinity.w*

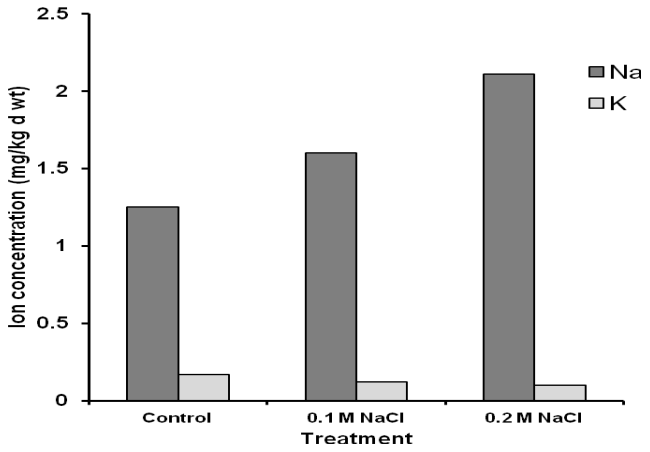

Figure. 8 *Sodium and potassium ion concentrations in shoot of Amaranthus hybridus treated with salinity.*

of 0.126 ±0.004 µmol ascorbate/mg protein/min. The control plants had 0.054 ±0.002 µmol ascorbate/mg protein/min, while plants that were treated with 0.1M NaCl had 0.092 ± 0.002 µmol ascorbate/mg protein/min (Fig.6). Figure 7 showed the protein contents of *A. hybridus* as affected by salinity stress. The total protein content decreased with increasing salinity. The least protein level was

observed in plants treated with 0.2M NaCl which had 0.19% protein compared to 0.31% observed in the control plants. Salinity also affected the concentrations of Na and K ions contents in *A. hybridus*. As expected, Na^+ significantly increased with increasing salinity, while K^+ decreased significantly (p=0.05) with increasing salinity (Fig.8).

4. Discussion

Growth inhibition by salt in *Amaranthus hybridus* was significant in this study, confirming the high sensitivity of this species to salinity. The reduction in biomass accumulation could be as a result of the high osmotic pressure of external solution that hindered root absorption of water and mineral nutrients. Similar results have been observed in *Phaseolus vulgaris* (Ferri *et al*., 2000) and *Bruguiera gymnorrhiza* (Takemura *et al*., 2000). The result of the study indicated a decrease in relative water content (RWC) in salinity stressed plants. This observation may be due to the increase in the osmotic pressure of the external solution created by high salinity which reduced the water potential and directly affect water uptake by plant roots. Through the analysis of water relations in the leaves of *A. hybridus*, we observed that plants adjusted their water potential to more negative levels as salinity increased (Fig. 2), which is a common reaction to salinity similar to those reported for other species (Qin *et al*., 2010; Redondo-Gomez *et al*., 2010; Suarez & Medina, 2008).

We observed a significant decrease in the level of total chlorophyll in the leaves of *A. hybridus* plants grown under saline conditions (Fig. 3). Chlorophyll concentration is often used to quantify leaf senescence in plants, and most studies have shown that salinity adversely affects chlorophyll content (Meloni *et al*., 2003). The decrease in chlorophyll content observed in this study may be attributed to both an inhibited synthesis of the pigment and damaged photosystem antenna caused by reactive oxygen species (Kato & Schimizu, 1985; Youssef & Awad, 2008). Salinity stress often induced the increase of reactive oxygen species (ROS), which destroy membranes through lipid peroxidation. As MDA is an end product of membrane lipid peroxidation, the concentration of MDA represents the degree of cell membrane damage under stress and is a common physiological indicator for evaluating plant exposed to either abiotic or biotic stress (Chen *et al*., 2000; Dogan *et al*., 2010). The result of this study indicated significant increase in MDA content in salinity stressed plants. The finding was in agreement with past investigations.

Stress increases formation of ROS and increase the activity of the enzymes that detoxify these species (Apel & Hirt, 2004; Foyer & Noctor, 2005). The coordinated activity of these antioxidant enzymes in the different cell compartments achieves a balance between the rate of formation and removal of ROS, and maintains hydrogen peroxide (H_2O_2) at the levels required for cell signaling (Munns & Tester, 2008). A significant increase in the activities of catalase and ascorbate peroxidase observed in this study may be related to the increased level of ROS formation due to salinity stress.

In this study, we observed that salinity decreased the total protein content in *A. hybridus*. A decrease in protein content with the increase of NaCl was also reported by Azooz *et al*. (2004) and Dager *et al*. (2004). In many plant tissues a reduced water potential causes a reduction of total protein synthesis and a rapid dissociation of polyribosomes (Bardzik *et al*., 1971). Salinity alters uptake and absorption rates of all mineral nutrients resulting in deficiency symptoms. Bonilla *et al*. (2004) found that most toxic effects of NaCl can be attributed to Na^+ toxicity. Excessive accumulation of Na^+ can cause a range of osmotic and metabolic problems for plants (Hoai *et al*., 2003). In the present study, Na^+ content in the leaves of salinity stressed plants increased by about 50 and 100 % compared with the control at 0.1 and 0.2M respectively (Fig. 8), which indicated that *A. hybridus* had no efficient capacity to restrict sodium movement to the aerial part of the plant. Potassium is an important plant nutrient that plays important roles in stomatal behavior, osmoregulation, and membrane polarization among others (Qin *et al.,* 2010). It was observed in this study that the salt treatments decreased K^+ content with increasing NaCl concentration (Figure 8). The observation reported here agreed with earlier reports by Wang *et al*., 2004; Qin *et al*., 2010; Nivas *et al*., 2011; Yue *et al.,* 2012. The observation may be attributed to the fact that Na^+ enters roots passively, via voltage-independent (or weakly voltage-dependent) nonselective cation channels (Amtmann *et al*., 1999) and possibly via other Na^+ transporters such as members of the high-affinity K^+ transporters (Haro *et al*., 2005; Laurie *et al*., 2002), therefore, increase in the concentration of external Na^+ will logically increased the rate at which Na^+ accumulate in the plant with concomitant decrease in K^+ uptake.

In conclusion, data from this study showed that salinity stress had significant effects on the physiological and biochemical parameters evaluated, confirming that salinity generally decreased crop productivity.

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