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INFLUENCE OF ARBUSCULAR MYCORRHIZAL FUNGI ON THE TOLERANCE OF PEPPER TO DROUGHT STRESS

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ABSTRACT

Pepper is an important spice widely cultivated in the world for food vegetables, medicine and other purposes. Climate change has resulted in seasonal variation in the amount of rainfall which has impacted negatively on food production, especially pepper, in Nigeria. This work attempts to enhance the growths and yields of pepper by investigating the influence of Arbuscular mycorrhizal fungi (Glomus and Gigaspora spp) on the tolerance of pepper to drought stress. This research was conducted at the Botanical Garden, Federal College of Education, Abeokuta, Ogun State, Nigeria. The experiment was laid out in complete randomized block design with five replications. Parameters measured include: stem height, number of leaves, number of branches and fruits number. Data obtained were subjected to ANOVA while means were separated by Duncan multiple range test (DMRT). Results shows that water-stressed Glomus mosseae treated plants had higher stem height, number of leaves, number of branches and fruits number among the water-stressed mycorrhizal plants. Other species of AMF also produced higher growth and yield related characters when compared with non-mycorrhizal plants. Conclusively, the results of this work implied that AMF have the potentials in influencing tolerance of pepper to drought stress. Glomus mosseae is considered the best species with higher drought tolerance potential for pepper.

KEYWORDS: Drought tolerance; arbuscular mycorrhizal fungi; growth, water-stressed.

INTRODUCTION

Pepper belongs to the genus *Capsicum* which consists of about 20-27 species, out of these, only five are domesticated, these are: *C. Annum, C. Baccatum, C.chinense, C. Frutescens, C.*

Pubescens (Walsh and hoot, 2001). The generic name – *Capsicum*, may come from latin word capsa which means 'box', presumably alluding to the pods. It may also come from the Greek word kapto which may be interpreted as 'to gulp (Umberto, 2000). The name "pepper" comes from the similarity of the flavour to black pepper, *piper nigrum*, although there is no botanical relationship with it, pepper grows well on a warm, moist loamy soil with temperature of about $21^{\circ}C - 25^{\circ}C$. Fruits of capsicum can vary tremendously in colour, shape and size both between and within species which led to confusion over the relationship among taxa (Eshbaugh, 1975). Capsicum has become cultivated worldwide and it has also become key element in many cuisines. In addition to use as spices and food vegetables, *Capsicum* has also been used as medicines and lachrymatory agents (Umberto, 2000).

Symbiotic interactions between plants and microorganisms is a beneficial approach to enhance plants' productivity and reducing environmental costs, that are mainly due to the massive use of inorganic fertilizers. Example of such association is that of arbuscular mycorrhizal fungi (AMF) which is an important group of soil microorganisms, which provide an increased interface between roots and soil, so improving the plant nutritional state, especially the phosphatic one and better tolerate biotic and abiotic stresses like drought and ameliorating fruit yield and quality (Hodge, Helgason, & Fitter, 2010). Cesaro *et al.* (2020) reported that there was no stress symptoms observed in the mycorrhizal plants and that the fungus behaved as a mutualistic symbiont during the early stages of plant growth but a trend towards the positive effects on plant growth was observed as the plants age in mycorrhizal plants.

AM fungi are capable of significantly improving plant mineral nutrient acquisition, mainly in low-nutrient conditions, and it has clearly been demonstrated that plants possess a symbiotic Pi uptake pathway (Harrison *et al.*, 2002; Bucher, 2007; Smith and Smith, 2011. Thus, AM fungi are primary biotic soil components which, when missing or impoverished, e.g., due to anthropic input, can lead to a less efficient ecosystem functioning. The process of re-establishing the natural level of AMF richness can represent a promising alternative to conventional fertilization practices, with a view to sustainable agriculture, a key target for growers facing the global recession and having to deal with a more environmentally aware clientele. The main strategy adopted to achieve this goal is the direct re-introduction of AMF propagules (inoculum) into a target soil. Furthermore, AM fungi can also have a direct effect on the ecosystem, as they improve the soil structure and aggregation (Rillig and Mummey, 2006; Leifheit *et al.*, 2014, 2015) and drive the structure of plant communities and productivity (van der Heijden *et al.*, 1998).

Pepper is a water loving plant hence adequate supply of water is required for its normal growth and yield. Availability of water makes pepper available and cheap during the raining season but scarce and expensive during the dry season. There is therefore, the need to develop a technology that will improve the supply and availability of pepper throughout the year and remove overdependence on water availability.

The objective of this work was to investigate the growth and yield responses and arbuscular mycorrhizal fungi potential in pepper's drought tolerance and to identify drought resistant pepper variety that can be cultivated all the year round.

METHODOLOGY

The research was carried out at the Botanical Gardens, Biology Department Federal College of Education, Abeokuta, Ogun State, South-western Nigeria. The experiment was laid out in a completely randomized design with five replicates. Pepper seeds which include drought susceptible varieties were obtained from the National Centre for Genetics Resources and Biotechnology, Ibadan, Oyo state, Nigeria. Arbuscular Mycorrhizal Fungi (AMF-*Glomus deserticola, Glomus clarum, Glomus mosseae and Glomus gigaspora)* were obtained from the Soil Microbiology Laboratory, Department of Agronomy, University of Ibadan, Oyo State. Soils collected from the plot of land behind the Botanical Gardens of the Biology department, Federal College of Education, Abeokuta, Ogun State were sterilized at the soil Laboratory and bagged into pots. Ten Seeds were planted per pots and thinned down to one per pot at 30 days after planting when the treatments were established. Treatments for the study are as presented in Table 1.

Some agronomic data studied in this research include; plant height, cumulative numbers of leaves per plant, cumulative number of fruits per plant were taken at the growth and yield stage.

| S/N | TREATMENTS | SYMBOLS |
|-----|--|-------------------------|
| 1. | Pepper alone + watering | PW^+ |
| 2. | Pepper alone + water stressed | PW⁻ |
| 3. | Pepper + AMF (Glomus deserticola) + water stressed | PGDW |
| 4. | Pepper + AMF (Glomus deserticola) + watering | PGDW+ |
| 5. | Pepper + AMF (Gigaspora gigantea) + watering | \mathbf{PGGW}^+ |
| 6. | Pepper + AMF (Gigaspora gigantea) + water stressed | PGGW |
| 7. | Pepper + AMF (Glomus mosseae) + watering | \mathbf{PGMW}^+ |
| 8. | Pepper + AMF (Glomus mosseae) + water stressed | PGMW ⁻ |
| 9. | Pepper + AMF (Glomus clarum) + watering | PGCW^+ |
| 10. | Pepper + AMF (Glomus clarum) + water stressed | PGCW ⁻ |

Table 1: Treatments



Plate 1: Water-stressed pepper

plate 2: Well-watered mycorrhizal pepper

RESULTS AND DISCUSSION

| | WEEKS AFTER TREATMENT | | | | | | | | |
|---------------------|-----------------------|--------------------|----------------------|----------------------|--------------------|----------------------|--|--|--|
| TREATMENTS | 6 | 8 | 10 | 12 | 14 | 16 | | | |
| \mathbf{PW}^+ | 57.00 ^e | 63.40^{f} | 65.80^{f} | $68.20^{\rm e}$ | $73.40^{\rm e}$ | $74.40^{\rm f}$ | | | |
| PW ⁻ | 52.60^{f} | 57.00 ^g | 59.50 ^g | 60.30 ^g | 61.30^{f} | 61.80 ^g | | | |
| \mathbf{PGDW}^+ | 76.40^{a} | 84.00^{ab} | 92.29 ^a | 96.60 ^a | 102.20^{a} | 105.00^{a} | | | |
| PGDW ⁻ | 63.60 ^c | 69.80^{d} | 80.00° | 80.14 ^c | 87.40° | 90.50^{d} | | | |
| \mathbf{PGGW}^{+} | 67.80^{b} | 85.20^{a} | 89.80^{a} | 91.60 ^b | 94.00^{b} | 100.20^{ab} | | | |
| PGGW ⁻ | 61.40^{d} | $65.60^{\rm e}$ | 71.00^{d} | 79.64 ^d | 87.40° | 90.10 ^d | | | |
| \mathbf{PGCW}^+ | 64.40 ^c | 73.60 ^c | 84.80^{b} | 79.04 ^d | 95.00^{b} | 100.75^{ab} | | | |
| PGCW ⁻ | 51.00^{f} | 59.20 ^g | 68.21 ^e | 65.38^{f} | 81.40^{d} | $89.40^{\rm e}$ | | | |
| \mathbf{PGMW}^+ | 62.00^{d} | 83.20 ^b | 88.60^{a} | 92.40^{b} | 100.80^{a} | 103.65 ^{ab} | | | |
| PGMW ⁻ | 44.70 ^g | 62.20^{f} | 72.20 ^d | 80.64 ^c | 89.80 ^c | 92.10 ^c | | | |

| Table 2: Plants' height of Capsicum chinense treated with Arbuscular Mycorrhizal Fungi | |
|--|--|
| (AMF) under different watering regime | |

Values are means of five replicates. Means with the same letter in a column are not significantly different (DMRT at p<0.05). PW⁺ = Well-watered pepper; PW⁻ = Water stressed pepper; PGDW⁺ = Well-watered pepper inoculated with *Glomus deserticola*; PGDW⁻ = Water stressed pepper inoculated with *Glomus deserticola*; PGGW⁺ = Well-watered pepper inoculated with *Glomus clarun*; PGCW⁻ = Water stressed pepper inoculated with *Glomus clarun*; PGCW⁻ = Water stressed pepper inoculated with *Glomus mosseae*; PGMW⁺ = watered pepper inoculated with *Glomus mosseae*; PGM⁺ = watered pepper inoc

The plants' height of Arbuscular mycorrhizal fungi inoculated Capsicum chinense under different water regime was presented in Table 2. Well-watered Pepper plants treated with Glomus deserticola (PGDW⁺) had the highest stem height (76.4 cm) at 6 weeks after treatment (WAT) while plants treated with *Glomus mosseae* (PGMW⁺) had the least (44.7 cm). At 8WAT, PGGW⁺ had the highest stem height (85.2 cm) which is significantly different from all the other treatments while water-stressed non-inoculated pepper plant had the least (57 cm). It was interesting to note that water-stressed AMF inoculated plant (PGDW⁻) had values greater than well watered non AMF inoculated plant (PW⁺). At 10WAT PGDW⁺, PGGW⁺ and PGMW⁺ had values which are not significantly different from each others. The non-inoculated water-stressed plant had the least value for stem height. From 12- 16WAT, well-watered pepper inoculated with Glomus deserticola (PGDW⁺) had the highest values for stem height, these values are significantly different from all the other treatments. Water-stressed Glomus mosseae treated plants (PGMW) had the highest values for stem height among the water-stressed AMF inoculated plants. This was in line with the report of Afolayan and Oyetunji (2016; 2018) that G. mosseae enhanced higher shoot production in plants. Water-stressed mycorrhizal plants had higher stem height than the non-mycorrhizal plants. Oyetunji and Afolayan, (2007) reported higher mycelia in arbuscular mycorrhizal fungi which assist the plants to absorb more water and mineral nutrients which would have been inaccessible to the plants. Also, Olawuyi et al. (2014) reported enhanced stem height in mycorrhizal plants when compared to its non-mycorrhizal counterpart. Afolayan, Adeniji and Muazu, (2015) reported that AMF influenced the stem height in beans.

TREATMENTS

| | 6 | 8 | 10 | 12 | 14 | 16 |
|---------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| \mathbf{PW}^+ | 13.20 ^a | 13.80 ^c | 14.70 ^d | 16.00 ^d | 16.00 ^e | 16.40 ^e |
| PW ⁻ | 11.40^{b} | $10.40^{\rm e}$ | 11.60 ^f | $12.10^{\rm e}$ | 12.50^{f} | $12.50^{\rm f}$ |
| \mathbf{PGDW}^{+} | 11.60 ^b | 15.60 ^a | 16.20^{b} | 20.60^{a} | 21.40^{a} | 22.00^{a} |
| PGDW ⁻ | 10.80° | 14.40^{b} | 15.70° | 20.20^{a} | 21.80^{a} | 22.40^{a} |
| \mathbf{PGGW}^+ | 13.40 ^a | 15.44 ^a | 18.30^{a} | 20.20^{a} | 20.80^{b} | 21.90^{b} |
| PGGW ⁻ | 11.20 ^b | 13.00° | 15.00° | 17.60° | 20.20^{b} | 20.40° |
| \mathbf{PGCW}^+ | 12.00^{ab} | 14.00 ^b | 15.00° | 18.80^{b} | 18.80° | 18.80^{d} |
| PGCW ⁻ | 11.60 ^b | 12.20 ^d | $13.40^{\rm e}$ | 17.20° | 17.60 ^d | 18.00 ^d |
| \mathbf{PGMW}^+ | 9.40 ^c | 11.68 ^d | $13.80^{\rm e}$ | 17.00° | 19.00° | 21.20 ^b |
| PGMW ⁻ | 7.60 ^d | 11.00 ^d | 14.60 ^d | 16.70 ^d | 18.40 ^c | 18.80 ^d |

WEEKS AFTER TREATMENT

Table 3: Number of Branches of Capsicum chinense treated with Arbuscular Mycorrhizal Fungi (AMF) under different watering regime

Values are means of five replicates. Means with the same letter in a column are not significantly different (DMRT at p<0.05). PW⁺ = Well-watered pepper; PW⁻ = Water stressed pepper; PGDW⁺ = Well-watered pepper inoculated with *Glomus deserticola;* PGDW⁻ = Water stressed pepper inoculated with *Glomus deserticola;* PGGW⁺ = Well-watered pepper inoculated with *Glomus clarum;* PGCW⁻ = Water stressed pepper inoculated with *Glomus clarum;* PGCW⁻ = Water stressed pepper inoculated with *Glomus mosseae;* PGMW⁺ = watered pepper inoculated with *Glomus mosseae;* PGM⁺ = watered peppep

Table 3 shows the effects of different regime of water on the number of branches of *Capsicum* chinense treated with AMF. Gigaspora gigantea inoculated, well-watered plants (PGGW⁺) produced the highest number of branches (13.4) at 6 WAT while water-stressed G. Mosseae treated plants had the least (7.6). But at 8WAT, there was no significant difference in the number of branches between well-watered and water-stressed plants treated with G. Mosseae. Wellwatered PGGW had the highest number of branches at 8, 10 and 12WAT. An interesting observation was recorded at 12WAT, well-watered PGDW⁺ and water-stressed PGDW⁻ had values which were not significantly different but were significantly different from nonmycorrhizal plants. This development continued until the end of the study. This is due to the high absorbing capacity for water and immobile nutrients elements of mycorrhizal mycelia. This corroborates the report of many workers (Read et al., 1991) that mycorrhizal fungi mycelium directly supplied a significant amount of water and nutrients to the host plants. The grave difference in the number of branches between mycorrhizal and non-mycorrhizal plants is a pointer to how AMF can minimize the effects of drought on some plants' produce and prevent scarcity. This was in line with the study reported by Afolayan, Adeniji and Muazu, (2015) that AMF inoculated beans had higher number of branches when compared with other treatments.

| TREATMENTS | | WEEKS AFTER TREATMENT | | | | | | | | | |
|-------------------|--------------------|-----------------------|-----------------------|--------------------|---------------------|---------------------|--|--|--|--|--|
| | 6 | 8 | 10 | 12 | 14 | 16 | | | | | |
| \mathbf{PW}^+ | $17.00^{\rm f}$ | $30.10^{\rm f}$ | 35.70 ^f | 38.30 ^g | 45.20^{f} | 41.20 ^g | | | | | |
| PW ⁻ | 15.30^{f} | 20.40^{g} | 29.90 ^g | 30.00^{h} | 35.80 ^g | 36.30^{h} | | | | | |
| \mathbf{PGDW}^+ | 41.20^{b} | 56.20^{ab} | 76.60° | 93.20 ^c | 110.40^{a} | 117.00^{b} | | | | | |
| PGDW ⁻ | 33.40 ^d | 49.50^{d} | 68.50° | 76.14^{f} | $89.00^{\rm e}$ | 93.10 ^f | | | | | |
| \mathbf{PGGW}^+ | 37.90 ^c | 55.60^{ab} | 80.00^{ab} | 96.20^{b} | 109.00 ^b | 119.50^{ab} | | | | | |
| PGGW ⁻ | 31.10 ^e | 45.60 ^e | 72.50^{d} | 81.60^{d} | 97.10 ^c | 99.70 ^d | | | | | |
| \mathbf{PGCW}^+ | 44.60^{a} | 63.40^{a} | 83.00 ^a | 99.00^{a} | 108.00^{b} | 116.20^{b} | | | | | |
| PGCW ⁻ | 37.90 ^c | 49.10 ^d | 65.71 ^e | 74.30^{f} | 91.20 ^d | 95.20 ^e | | | | | |
| \mathbf{PGMW}^+ | 42.00^{b} | 63.80 ^a | 84.20^{a} | 98.00^{a} | 112.80^{a} | 123.05 ^a | | | | | |
| PGMW ⁻ | 34.20^{d} | 52.60 ^c | 74.00° | 79.04 ^e | 94.10 ^c | 107.10° | | | | | |

| Table 4: | Cumulative Number | of l | leaves | of | pepper | treated | with | AMF | under | different |
|----------|--------------------------|------|--------|----|--------|---------|------|-----|-------|-----------|
| | watering regime | | | | | | | | | |

Values are means of five replicates. Means with the same letter in a column are not significantly different (DMRT at p<0.05). PW⁺ = Well-watered pepper; PW⁻ = Water stressed pepper; PGDW⁺ = Well-watered pepper inoculated with *Glomus deserticola;* PGDW⁻ = Water stressed pepper inoculated with *Glomus deserticola;* PGGW⁺ = Well-watered pepper inoculated with *Gigaspora gigantea;* PGGW⁺ = Water stressed pepper inoculated with *Gigaspora gigantea;* PGGW⁺ = Well-watered pepper inoculated with *Glomus clarum;* PGCW⁺ = Well-watered pepper inoculated with *Glomus clarum;* PGMW⁺ = well-watered pepper inoculated with *Glomus mosseae;* PGMW⁺ = watered pepper inoculated with *Glomus mosseae.*

Table 4 shows the cumulative number of leaves of pepper treated with AMF under different watering regime. At 6WAT, well-watered plants treated with G. *clarum* (PGCW⁺) had the highest number of leaves (44.6), while watered-stressed non-mycorrhizal plants (PW⁻) had the least (15.3). Similar observations were made at 8, 10 and 12 with PGCW⁺ having higher values which are not significantly different from PGMW⁺ but were different from all the other treatments. Plats treated with PGMW⁺ had the highest values at 14 WAT and 16WAT. There is no significant difference in the cumulative number of leaves between PGDW⁺, PGGW⁺ and PGCW⁺. This was in line with the work of Hata, Kobae, and Bamba, (2010) who opined that AMF improved soil structure may also trigger plant growth and development Well-watered non-mycorrhizal plants had the least values throughout the time of the experiment.

| TREATMENTS | | WEEKS AFTER TREATMENT | | | | | | | | | |
|---------------------|----------------|-----------------------|---------------------|--------------------|--------------------|--------------------|--|--|--|--|--|
| | 10 | 12 | 14 | 16 | 18 | 20 | | | | | |
| \mathbf{PW}^+ | 0.00^{d} | $3.00^{\rm e}$ | 5.00^{f} | 8.80 ^g | $15.60^{\rm f}$ | 41.00 ^g | | | | | |
| PW ⁻ | 0.00^{d} | $2.00^{\rm e}$ | 2.60^{g} | 5.00^{h} | 9.00 ^g | 15.00 ^h | | | | | |
| \mathbf{PGDW}^{+} | 4.00^{b} | 8.00^{d} | 13.20 ^e | $20.10^{\rm e}$ | 38.30 ^b | 57.00^{b} | | | | | |
| PGDW ⁻ | 3.00° | 8.00^{d} | 10.50° | 16.04^{f} | $24.00^{\rm e}$ | 30.10^{f} | | | | | |
| \mathbf{PGGW}^+ | 7.90^{a} | 15.30^{a} | 20.00^{a} | 25.90^{d} | 37.20 ^b | 52.00^{d} | | | | | |
| PGGW ⁻ | 3.10° | 10.00° | 14.60^{d} | $21.20^{\rm e}$ | 30.00° | $34.00^{\rm e}$ | | | | | |
| \mathbf{PGCW}^+ | 4.00^{b} | 13.70^{ab} | 19.50^{ab} | 28.00° | 44.00^{b} | 67.10^{a} | | | | | |
| PGCW ⁻ | 3.00° | 9.00 ^d | 12.01 ^e | 25.20^{d} | 30.30 ^d | 55.30 ^c | | | | | |
| \mathbf{PGMW}^{+} | 4.00^{b} | 13.00^{ab} | 21.70^{a} | 36.00^{a} | 48.20^{a} | 67.05^{a} | | | | | |
| PGMW ⁻ | 3.00° | 10.00° | 16.00° | 30.04 ^b | 40.10° | 53.50 ^d | | | | | |

| Table 5: | Cumulative | Number | of fruits | pepper | treated | with A | AMF | under | different | water |
|----------|------------|--------|-----------|--------|---------|--------|-----|-------|-----------|-------|
| | regime | | | | | | | | | |

Values are means of five replicates. Means with the same letter in a column are not significantly different (DMRT at p<0.05). PW⁺ = Well-watered pepper; PW⁻ = Water stressed pepper; PGDW⁺ = Well-watered pepper inoculated with *Glomus deserticola;* PGGW⁺ = Well-watered pepper inoculated with *Gigaspora gigantea*; PGGW⁻ = Water stressed pepper inoculated with *Gigaspora gigantea*; PGGW⁺ = Well-watered pepper inoculated with *Glomus clarum;* PGCW⁻ = Water stressed pepper inoculated with *Glomus clarum;* PGGW⁺ = Well-watered pepper inoculated with *Glomus clarum;* PGGW⁺ = Well-watered pepper inoculated with *Glomus clarum;* PGGW⁺ = Well-watered pepper inoculated with *Glomus clarum;* PGMW⁺ = Well-watered pepper inoculated with *Glomus mosseae*; PGMW⁺ = watered pepper inoculated with *Glomus mosseae*

The cumulative number of fruits of pepper treated with arbuscular mycorrhizal fungi under different watering regime was observed and presented in Table 5. Fruits were observed at 10WAT except in the non-mycorrhizal pepper plants. Plants treated with *Gigaspora gigantea* and well watered (PGGW⁺) had enhanced number of fruits at 10WAT. PGGW⁺ had higher cumulative number of fruits at 12WAT (15.3) which was not significantly higher than PGCW⁺ and PGMW⁺ but were significantly different from the other treatments while PW⁻ had the least (2.0). Observation at 14WAT was a bit different from the previous weeks in that PGMW⁺ had the highest cumulative number of fruits (21.7) but this value was not significantly different from PGGW⁺. At 16WAT and 18WAT PGMW⁺ had value that are significantly higher than all the other treatments while at 20WAT, PGMW⁺ and PGCW⁺ had higher values that were not significantly different from each other but were different from other treatments.

The cumulative number of fruits was higher in PGCW⁻ (55.3) than PGGW⁺ (52.0). Waterstressed *G. mosseae* (PGMW⁻) treated plants had cumulative number of fruits that are not significantly different from that of well-watered *Gigaspora gigantea* treated plants (PGGW⁺). Glomus mosseae and Glomus Clarum better adapted to water-stressed and performed better in terms of number of fruits produced during this period. The results of this study was similar to report of Afolayan and Oyetunji, (2017; 2018) who opined that AMF enhanced higher roots and tuber production in white yam and white yam vine cuttings. This higher production of fruits in both watered and water-stressed mycorrhizal plants might be as a result of high absorptive surface area of mycorrhizal plants as reported in the work of Oyetunji, Ekanayake and Osonubi (2003). Arbuscular mycorrhizal has been shown to increase the productivity of a variety of agronomic crops (Sylvia, 1993). In a related study, Afolayan, Oyetunji, Olawuyi and Ajanlekoko, (2017) reported that AMF influenced pepper yield when planted on spent engine oil.

CONCLUSION

Pepper is an important crop that is consumed daily in Africa and especially Nigeria. Its production all year round is constraints by seasonal rainfall. This accounts for unstable price and scarcity during the dry season. Mycorrhizal fungi have proved effective in enhancing tolerance of pepper to water stress. The use of arbuscular mycorrhizal fungi (AMF) will help to alleviate peppers' shortages and scarcity during the off-season. Glomus mosseae has higher potential to tolerate water stress in pepper and its thereby recommended.

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