Int J Seed Spice, 14(1), June, 2024: 65-72 DOI: https://doi.org/10.56093/IJSS.v14i1.7

Water deficit stress condition alters stress metabolites and essential oil content of coriander (*Coriandrum sativum* L.)

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Received: 23 October 2024
Revision: 08 November 2024
Accepted: 11 December 2024

Citation
Yadav, S.N., Mittal, G.K., Singh, B., Manohar, P. and Mahatma, M.K. 2024. Water deficit stress condition alters stress metabolites and essential oil content of coriander (*Coriandrum sativum* L.). *Int J Seed Spice,* 14(1):65-72

DOI
https://doi.org/10.56093/IJSS.v14i1.7

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Abstract
Changes in relative water content (RWC), stress metabolites and essential oil (EO) content were studied in five genotypes of coriander during water deficit stress at 70 days after sowing (DAS) and 90 DAS. Leaf samples were taken for RWC and stress metabolites analysis while seeds were taken for estimation of EO content. A significant reduction in RWC was observed in all the genotypes at both the stages (*viz* 70 DAS & 90 DAS) critical to water deficit stress. Coriander genotype UD-07 and UD-31 had lowest reduction, it can be inferred that genotype UD-07 had best tolerated to water deficit stress condition followed by UD-31. Induced water deficit stress declined membrane stability index with concomitant increase in the lipid peroxidation product malondialdehyde in all genotypes at both stages. Genotypes UD-07 and UD - 31 were found to have higher stability indices and a significantly lower accumulation of the malondialdehyde (MDA). Accumulation of osmo-protectant L-proline was significantly increased in all genotypes at both stages, however highest induction was observed in genotype UD-07 and UD-31. EO content was increased in all genotypes under water deficit stress; however it was slightly increased in genotypes UD-07 and UD-31. These results showed that genotype UD-07 and UD-31 had better physiological and biochemical mechanisms to combat water deficit stress.

Key Words: Coriander, metabolites, water stress,.

Introduction
Coriander (*Coriandrum sativum* L.) belongs to Apiaceae family is a major seed spice crop grown in India since ancient times. The entire plant at young stage is used in preparing chutneys and leaves are used for flavoring curries, souces, soups and are added to the food for flavoring, seasoning and imparting aroma or to improve its appearance, flavor and taste that increase our appetite. Dry seeds are used in preparation of curry powder and garam masala and seed oil used in industries for flavoring the products, like pastry, cookies,

cakes, buns, candy, cocoa, chocolate, confectionery, cordial, soda, syrups, preservers, gelatin dessert, tobacco and alcoholic beverages, particularly the 'gin'.

Coriander is mainly a crop of tropics and sub-tropics. The major producing countries other than India are Morocco, Russia, Bulgaria, Mexico, Argentina, China, Romania, Japan and Italy. In India, coriander is largely cultivated in Madhya Pradesh, Gujarat, Rajasthan, Andhra Pradesh, Tamil Nadu, Orissa, Uttar Pradesh, and Uttaranchal states. In India the total production was 974 (In 000 MT) MT covering the area of 711 (In 000 ha) with a productivity 1.4 MT ha^{-1} (Spices Board, India and Ministry of Agriculture and Farmers Welfare, Govt. of India (2024) (on 3854 & past Issues).

Rajasthan stands third both in area and production after Madhya Pradesh and Gujarat in the coriander crop. In Rajasthan, coriander is mainly cultivated in Kota, Jhalawar, Baran, Bundi, Chittorgarh and Udaipur districts. If we look at the productivity of this crop we will realise that it is very low. Some of the very obvious reasons for such low yield are that this crop is often cultivated on marginal lands with poor management of soil fertility, irrigation, fertilizer, disease and pests.

Plants response to water stress conditions is accompanied by a variety of physiological and biochemical changes at cellular levels, thus making it a complex phenomenon (Shao *et al*., 2009). It is well known that drought stress brings about numerous metabolic, biochemical and physiological changes in plants like growth (Ashraf and Iram, 2005; Benjamin and Nielsen, 2006, Changan *et al*., 2018), water status, membrane stability (Bai *et al*., 2006, Changan *et al*., 2023), Drought stress disturbs water relation of plant and reduces leaf size, stem extension and root proliferation (Farooq *et al*., 2009; Shao *et al*., 2009). The impact of drought stress on plant metabolism is not only dependent on severity, time and duration of stress but also on the tolerance level of the respective plant species as well as on the cultivars or varieties (Reddy *et al*., 2004, Reddy *et al*., 2024). Therefore, present investigation was carried to characterize coriander genotypes based on RWC, membrane stability and stress metabolites and understanding of water stress tolerance mechanism.

Materials and Methods

Plant materials

The seeds of five genotypes (UD-07, UD-19, UD-21, UD-31, and UD-706) of coriander were procured from Incharge, AICRP on spices, Department of Plant Breeding and Genetics, Sri Karan Narendra College of Agriculture, Jobner, Rajasthan. These five genotypes were raised at Instructional farm, Sri Karan Narendra Collage of Agriculture, Jobner during Rabi season of 2015-2016.Coriander genotypes were sown in 3m x 3m plot, keeping row to row distance (30 cm x 30 cm) and plant to plant (10 cm x 10 cm) distance. Crop was sown on 7 November 2015, standard package of practices were followed during cropping period. The crop was irrigated regularly to maintain plant growth and development. The sowing was done in randomized block design with four replication and five genotypes in three sets. Among these, two sets were used for stress by withholding irrigation and remaining set was used as control by providing the normal irrigation.

Stress was imposed by withholding irrigation after 70 days and 90 DAS. The following analysis was done at 20 days after imposition of water stress *i.e*. 90 DAS and 110 DAS at both stages. Upper third leaf samples from both unstressed and stressed plants were used for RWC, membrane stability index and stress metabolites analysis. Essential Oil content was extracted from dry coriander seeds.

Relative Water Content (RWC): Fresh weight of the coriander leaves were taken and then kept in distilled water for 4 hour to obtain turgid weight (Barrs and Weatherly, 1962). The turgid weight was recorded after blotting the excess water on the surface of the leaf. Dry weight was obtained after drying the leaf in an oven at 60°C. The RWC was calculated by the formula (RWC % = Fresh weight-Dry weight/Turgid weight-Dry weight X 100 given by Slavik (1974).

Membrane stability index: The procedure described by Premchandra *et al.* (1990) and modified by Sairam (1994) was used for membrane stability index. Leaf sample (0.5 g) was placed in distilled water (50 ml). One set was kept at 40°C for 30 minutes and its conductivity (C_1, for electrolytic leakage) was recorded using conductivity meter. The second set was kept in boiling water bath at 100°C for 10 minutes and its conductivity (Cl_2) was recorded after cooling at room temperature. The MSI was calculated according to the formulae: MSI% = (1-C1/C2) x 100.

Stress metabolites

Malondialdehyde: Fresh leaf samples (0.2 g) were extracted in 5.0 ml of 6% trichloroacetic acid (TCA) solution centrifugation at 8000 rpm for 10 minute. Supernatant were collected. To 1 ml of the supernatant taken in a clean, dry test tube, was added 2.0 ml of Thio-Barbituric Acid (TBA) reagent mixed and incubated for half an hour in a boiling water bath. The tubes were than cooled to room temperature. The assay mixture was then centrifuged at 5000 rpm for 10 min. and clearing supernatant bearing yellow to light orange colour was read on spectrophotometer at two wavelength *viz.* 532 nm for MDA) and 600 nm for interfering substance (Heath and Packer, 1968). Millimolar concentration of MDA was calculated as follows: MDA (mM) = (O.D.532 –O.D. 600) x 155 (extinction coefficient)

L-Proline content: Proline content was determined following the method described by Bates *et al.* (1973). Fresh leaf sample (0.2 g) were extracted in 5.0 ml of 3% sulphosalicylic acid (SSA) using mortar-pestle at room temperature. The homogenates were centrifuged at 8000 rpm for 10 minutes. The clear supernatants were collected in separate test tubes. To 1.0 ml of supernatant was added 2 ml of glacial acetic acid and mixed thoroughly, followed by 2.0 ml acid ninhydrin reagent was added and mixed well. The test tubes containing assay mixtures were incubated in a boiling water bath for an hour and then cooled to room temperature. Four ml of toluene solvent was added to each tube and mix well using vortex mixture. Toluene fractions were collected and intensity of pink colour was read at 520 nm on a spectrophotometer. A standard curve was prepared using L-Proline (0.1 mg/ ml).

Essential Oil (EO) Content: Fifty gram of coriander seeds were pulverized and transferred to 1 litre flask of Clevenger type apparatus to extract essential oil. Distilled water approximately 400 ml was added to fill the flask calculated half full. Distillation is continued until two consecutive reading taken at one hour interval show no change in oil content.

Statistical analysis: All the observation was taken in each genotypes, replications and sets. The data was statistically analysed using Factorial Randomized Block Design (FRBD) according to Panse and Sukhatme, 1985.

Results and Discussion

Relative Water Content: During water stress leaf water content at both the stages in the 5 genotypes reduced from 9.21 % to 47.06% (Fig.1). Genotype UD-21 and UD 706 showed maximum reduction in water content. Genotypes UD-07 and UD-31 had lowest reduction (14.24, 18.62% at 70 DAS & 9.21 & 10.69% at 90 DAS), it can be inferred that genotype UD-07 showed best tolerance to water deficit stress condition followed by UD-31.

Membrane Stability Index: The values for stability of cellular membrane in the coriander genotypes revealed that there was decline in MSI percent of stressed plant in all genotypes at both stages. The MSI values varied from 52.55 to 60.99 percent on fresh weight basis under control, while under stress it varied from 47.15 to 57.89 percent on fresh weight at 70 DAS. Likewise at 90 DAS, MSI values varied 43.88 to 60.25 percent on fresh weight basis under control, while under stress the MSI values varied from 37.81 to 55.65 percent on fresh weight basis. The difference in degree of lowering of MSI values was found significant when both the stages were compared. Thus, the study showed significant decrease in MSI due to water stress at both the stages. The minimum decrease due to water stress was observed in genotype UD-07 (5.07%) at 70 DAS as well as 90 DAS (6.12%). The decrease was slightly higher at 90 DAS than at 70 DAS in all the genotypes (Fig 2).

Malondialdehyde: Stress induced membrane damage has been biochemically marked by the presence of MDA, as one of the thiobarbituric acid reducing substances (TBARS) that accumulates as a consequence of membrane lipid peroxidation. The MDA values in water deficit stressed plants were found higher over respective controls at both stages in all the genotypes. The content of MDA was higher at 70 DAS than 90 DAS, in all genotypes. MDA content varied from 17.17 to 34.72 m moles g^{-1} fresh weight under control, while under stress it varied from 26.90 to 37.39 m moles g^{-1}fresh weight at 70 DAS. Likewise at 90 DAS, MDA content varied from 16.08 to 23.91 m moles g^{-1} fresh weight under control conditions, while under stress the MDA content varied from 17.86 to 31.85 m moles g^{-1} fresh weight (Table-1). The present investigation showed that the MDA content increased minimum in genotype UD-07 (7.69%) at 70 DAS and at

90 DAS (9.09%) followed by genotype UD-31 (12.35%) at 70 DAS and at 90 DAS(11.08%). These results showed better stability of membranes in UD-07 and UD-31 genotypes. Conversely, genotype UD-706 (36.18 at 70 DAS, 33.23 at 90 DAS) had less membrane stability due to higher accumulation of MDA content at both stages.

L-Proline: The proline content varied from 30.34 to 47.95 mg 100^{-1}gfresh weight under control, while under stress it varied from 48.82 to 64.61 mg 100^{-1}gfresh weight at 70 DAS (Table 1). Likewise at 90 DAS, proline content increased and varied from 71.77 to 108.72 mg 100^{-1}gfresh weight under control, while under stress the proline content varied from 116.59 to 160.23 mg 100^{-1}gfresh weight. The present study showed significant increase in proline content due to water stress at both the stages. Proline content was increased more than two fold due to water stress in genotype UD-07 (112.95%) at 70 DAS and at 90 DAS (123.25%).

Essential Oil (EO) content: EO content was significantly increased in all the genotypes at both the stages *i.e.* 70 DAS & 90 DAS under water deficit stress condition. The accumulation of EO/volatile oil content was varied from 0.359 to 0.411% under control, while under stress it varied from 0.363 to 0.420% at 70 DAS. Likewise, at 90 DAS, volatile oil content varied from 0.365 to 0.440% under stress (Table-2).The maximum volatile oil of 0.411% was observed in UD-07 followed by 0.405% in UD-706 under control. Higher accumulation of volatile oil (0.420% at 70 DAS and 0.440% at 90 DAS) was observed in genotype UD-706. The percent increase of volatile oil in stress condition was noticed in genotype UD-21 as 4.80% at 70 DAS while at 90 DAS in genotype UD-706 (8.64%).

Water stress causes a wide array of biochemical and physiological changes, starting with a decrease in osmotic potential at the cellular level (Bajji *et al.*, 2001). The leaf water content at both the stages in the five genotypes indicates, on average ranges of reduction, 9.21 % - 47.06% during water stress. Genotypes UD-07 and UD-31 had lowest reduction (14.24, 18.62% at 70 DAS & 9.21 & 10.69% at 90 DAS). Thus it can be inferred that genotype UD-07 had best tolerance to water deficit stress condition followed by UD-31. Reactive oxygen species are known to damage cellular membranes by inducing lipid peroxidation (Devi *et al.*, 1998). Membrane stability index may be used as parameter to estimate the cellular injury caused to membrane due to peroxidation of fatty acids of the membrane and the levels of membrane lipid peroxidation can be measured by Thiobarbituric acid substance called malondialdehyde content. In present study, the increased levels of MDA in stress condition indicate the membrane sensitivity/ membrane damage to water stress. Lower rate of increase of MDA in genotypes indicates better membrane strength. The MSI reductions were found lowest in genotypes UD-07 and UD-31 at both the stages that implied water deficit stress tolerance capacity of these genotypes. Maximum reduction in MSI values was observed in genotype UD-21 and UD-706 at both stages, indicating their high susceptibility to water stress. The results are supported by Pant *et al.*, 2014, Mittal *et al.*, 2006, Mittal, 2010, Karmakar *et al.*, 2014, Meena *et al.*, 2016. In order to maintain osmotic balance, specific types of organic molecules accumulate in the cytoplasm which are termed as compatible solutes *e.g.* proline, ascorbic acid and glycine betaine These solutes do not impair normal physiological functions even if accumulated at high concentrations. The results of present investigation showed higher accumulation of L-proline at 90 DAS compared to 70 DAS in the stressed tissues of all genotypes indicates 90 DAS stage to be a more responsive stage in terms of cellular osmotic adjustment. Comparing performance of genotypes at the two stages, UD-07 and UD-31 were found to be tolerant. Likewise, genotypes UD-21 and UD-706 at both the stages exhibit high susceptibility to water stress amongst all five genotypes. EO content was increased all the genotypes due to water stress at both the stages. However, percent increase of EO content was less in UD-07 and UD-31 compared to other genotypes that further confirm the stability of biochemical machinery of these genotypes under water deficit stress conditions. The complete irrigation treatment produced better seed yield thus more essential oil yield. Other research has demonstrated an increasing of seed oil and essential oil percentages respectively in *Calendula officinalis* (Rahmani *et al.* 2008) and *Ocimum sp.* (Khalid 2006) and a decreasing essential oil yield of *Pelargonium graveolens* (Putievsky *et al.*, 1990) in drought stress conditions. In the control, essential oil yield in *C. cyminum* seeds was 1.64%, based on dry matter weight, and is affected by water treatment. Under MWD, this yield was 1.40 times higher in comparison to the control. Similarly, Bettaieb *et al*. (2011) found that the volatile oil percentage of *C. cyminum* aerial parts was greater in plants grown at MWD than the control ones. An increase of essential oil yield under a moderate degree of water deficit has been reported earlier in other plants, e.g. *Pimpinellaanisum* L. (Zehtab-Salmasi *et al*., 2001), *Petroselinum crispum* (Petropoulos *et al*., 2008) and C. carvi L. (Laribi *et al*., 2009).

Conclusions: The genotypes UD-07 and UD-31 showed higher water content, membrane stability and osmo-protectant compound which reduce membrane lipid peroxidation and maintain the EO content during water deficit stress conditions. Thus, these genotypes may be used to develop drought-tolerant coriander verities exploiting genetic engineering techniques.

Conflicts of interest: There are no conflicts of interest.

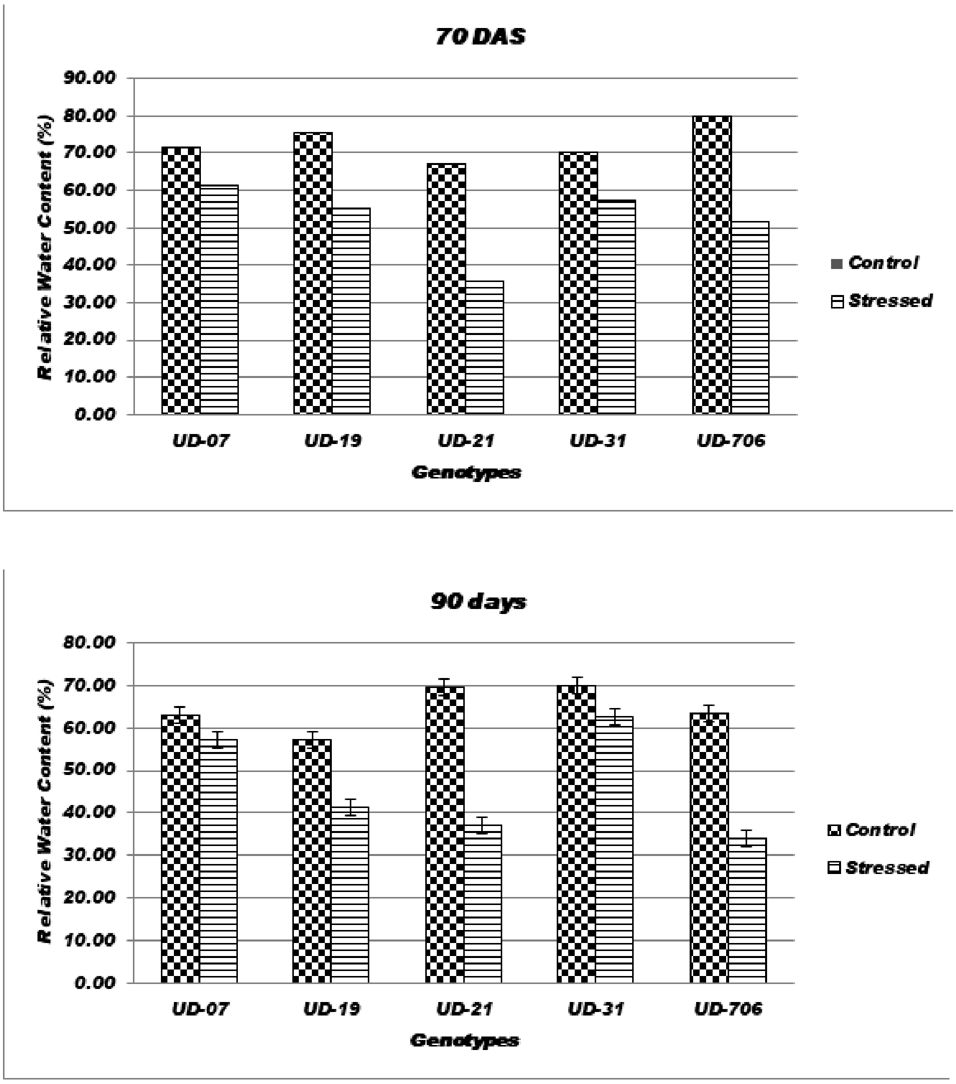

Fig. 1: Effect of water stress on relative water content in coriander genotypes at two stages. Analysis was done at 20 days after imposition of water stress at both stages, Error bar displayed CD value at 5% significance.

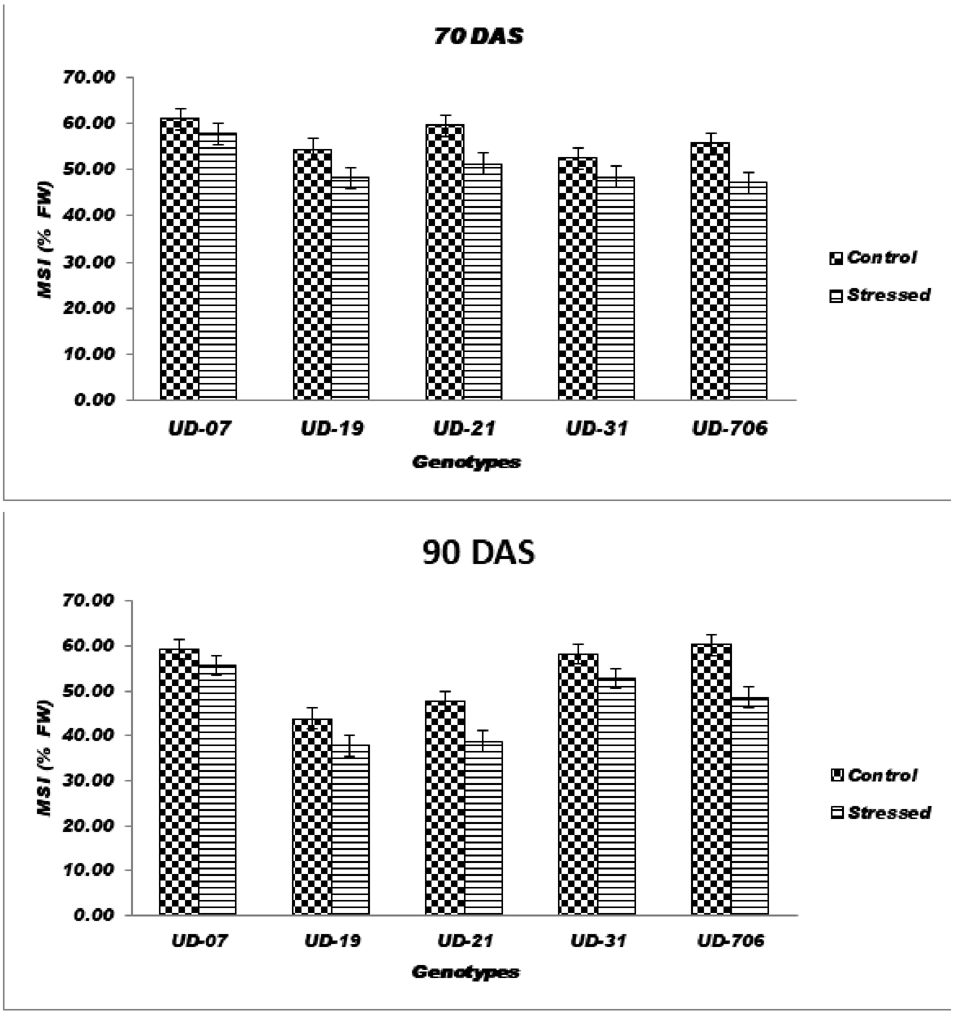

Fig. 2: Effect of water stress on membrane stability index in coriander genotypes at two stages. Analysis was done at 20 days after imposition of water stress at both stages, Error bar displayed CD value at 5% significance.

Table 1. Effect of water stress on Malondialdehyde and L-proline in coriander genotypes at two stages.

Genotypes	Comparative MDA content (m moles g^{-1}FW)						Comparative L-Proline content (mg $100g^{-1}$ FW)					
	70 DAS		% Incr.	90 DAS		% Incr.	70 DAS		% Incr.	90 DAS		% Incr.
	Control	Stressed		Control	Stressed		Control	Stressed		Control	Stressed	
UD-07	34.72	37.39	7.69	16.62	18.14	9.09	30.34	64.61	112.95	71.77	160.23	123.25
UD-19	25.89	35.28	26.63	20.42	24.88	21.8	43.68	53.05	21.45	95.86	124.98	30.37
UD-21	23.21	35.18	34.02	19.26	24.03	24.7	42.81	48.82	14.03	94.24	117.90	25.10
UD-31	29.49	33.64	12.35	16.08	17.86	11.1	35.88	60.61	68.92	81.46	142.66	75.12
UD-706	17.17	26.90	36.18	23.91	31.85	33.2	47.95	49.85	3.96	108.72	116.59	7.23
S. Em	0.37			0.23			0.71			1.49		
C. D. at 5%	1.07			0.66			2.05			4.30		

Analysis was done at 20 days after imposition of water stress at both stages.

Table 2. Effect of water stress on volatile oil content (%) in coriander genotypes at two stages.

Genotypes	Days at which water stress was applied					
	70 DAS			90 DAS		
	Control	Stressed	Percent Increase	Control	Stressed	Per cent Increase
UD-07	0.411	0.418	1.70	0.411	0.425	3.40
UD-19	0.385	0.400	3.89	0.385	0.413	7.27
UD-21	0.375	0.393	4.80	0.375	0.400	6.66
UD-31	0.359	0.363	1.11	0.359	0.365	1.67
UD-706	0.405	0.420	3.70	0.405	0.440	8.64
S. Em	0.004			0.003		
C. D. at 5%	0.012			0.010		

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