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STRESS INDUCED BIOCHEMICAL ALTERATION OF PROLINE IN PIGEONPEA - SMALL MILLETS - PEANUT BASED RAINFED CROPPING SYSTEM

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ABSTRACT

A field experiment was conducted during the *rabi* season of 2015 - 2016 at Dry land Agricultural Research Station, TNAU, Chettinad, Tamil Nadu. There were 45 combinations comprising of different cropping system, nutrient management and stress management practices with 2 replications. Results indicated that Pigeon pea + foxtail millet + Groundnut (1:2:1) + 100% RDF for base crop + foliar spray of 2% DAP +1% KCl + foliar spray of PPFM @500 ml ha^{-1} were significantly higher proline content in pigeon pea (52.85 and 64.85 mg g^{-1} FW), small millets (12.05 and 12.65 mg g^{-1} FW) and peanut (3.15, 3.45 mg g^{-1} FW) for both pre flowering and flowering stage of the crops.

KEYWORDS: Proline, Stress management, Nutrient management, Cropping system

INTRODUCTION

Plants can cope with drought stress through genetic and adaptive mechanisms. Plants possess the mechanisms to escape, avoid and/or resist to drought. They can also escape drought by adjusting their physiological and biochemical development according to the availability of water in their habitat (Arraudeau 1989). Water stress induces a significant decrease in metabolic factors such as decrease in chlorophyll content and enhanced accumulation of proline (Din *et al.* 2011). Accumulation of proline is a widespread plant response to environmental stress, including low water potential. Proline is an organic osmoprotectant accumulates in a large number of plant species exposed to environmental stresses such as salinity, drought, extreme temperature, UV radiations and heavy metals (Hare and Cress, 1997). Proline acts as a `compatible solute`, i.e. one that can accumulate to high concentration in the cell cytoplasm without interfering with cellular structure or metabolism (Yency *et. al.*, 1982 and Samaras, *et. al.*, 1995). Proline accumulation is believed to play adaptive roles in plant stress tolerance (Ashraf and Fooland, 2007).

India is the largest producer (25% of global production), consumer (27% of world consumption) and importer (14%) of pulses in the world. Pulses account for around 20 per cent of the area under food grains and contribute around 7-10 per cent of the total food grains production in the country. The area under pulses has increased from 19 million ha. in 1950-51 to 25 million ha. in 2013-14, indicating an increase of 31 per cent whereas the production of pulses during the same period has increased from 8.41 million ha. to 19.27 million ha. In India, there is an increasing demand especially for pulses and oil seeds besides cereals to cope up with increasing human population and to overcome malnutrition in the large section of society. Also per capita availability of pulses per day had come down from 69 g in 1961 to about 37.8 g in 2001. But, the per capita recommendation of WHO/FAO is 80 g per day. To bridge this gap, there is an urgent need to increase the pulse production. Since, there is a limited scope for increasing pulse production by increasing its area can be met by increasing the productivity through adopting agronomic practices of which intercropping system is one of the best ways to increase the production. (Rathod *et al.*, 2004).

MATERIALS AND METHODS

The experiment was conducted at Dry land Agricultural Research Station, Chettinad, Sivaganga District, Tamilnadu, India during rabi-2015-2016. The experimental site is located at 10^{o}10'N latitude and 78^{o}47' E longitude at an elevation of 126 m above Mean Sea Level. The farm is situated in the Southern Agro climatic Zone of the Tamil Nadu. The normal weather conditions of the location are as follows (mean of 31 years from 1980 to 2011). A mean annual rainfall of 894.4 mm. Out of which, 338 mm was distributed during South West Monsoon (SWM), 416.5 mm during North West monsoon (NWM), and 38.8 mm during winter and 101.1 mm during summer. The average maximum and minimum temperatures during cropping period were 25.67°C and 23.74°C respectively. The total rainfall during cropping period is 374.90 mm with the mean relative humidity of 81.26 and 83.91 per cent. The experiment was laid out in split-split plot design. There were 45 treatment combinations comprising of different nutrient and stress management practices with replicated twice. In treatments main plot consist of three different cropping system viz., M_1 – Pigeon pea (Co Rg.7) + barnyard millet (Co Kv.2) + Groundnut (VRI-2) (1:2:1), M_2 – Pigeon pea + foxtail millet (Co Te7) + Groundnut (1:2:1) and M_3 – Pigeon pea + kodo millet (Co3) + Groundnut (1:2:1) in sub plot consist of five nutrient management viz., N_1 – 100% RDF for base crop + no fertilizers for intercrops, N_2 – 100%RDF for all combination (both base and intercrops), N_3 – 100% RDF for base crop + 50% RDF for inter crops, N_4 – 100% RDF for base crop + foliar spray of 2% DAP +1% KCl and N_5 – 100% RDF for base crop + 50%RDF for intercrops+ foliar spray of 2% DAP +1%KCl and in sub sub plot three kind of stress management viz., S_1 – control, S_2 – foliar spray of PPFM @500 ml ha^{-1} and S_3 – foliar spray of 500 ppm cycocel (CCC). The pigeon pea was sown at a spacing of 120cm x 30 cm, the in between row of pigeon pea, minor millets and groundnut was sown in the proportion of 1:2:1 ratio. The recommended dose of fertilizer was given to pigeon pea i.e., 12.5:25:12.5 kg ha^{-1} in the form of urea and diammonium phosphate as a basal dose. The foliar spray were taken at pre flowering and flowering stage of the crops. Plant protection and weeding measures were taken as per their need. The crop was protected by spraying of chemicals to control pest and diseases. The proline content of the leaf was estimated as per the procedure suggested by Bates *et al.*, (1973) and expressed as mg g^{-1}.

RESULTS AND DISCUSSIONS

Red Gram (Table 1 & 2)

Among the multi-tier cropping system the results revealed highest proline content in pigeon pea was observed in (M_2) Pigeon pea + foxtail millet + Groundnut (1:2:1) followed by (M_3) Pigeon pea + kodo millet + Groundnut (1:2:1) (44.95, 51.74 and 43.50, 50.10 mg g^{-1} FW), in both pre flowering and flowering stage. With regard to nutrient management significantly highest proline content was observed in the treatment of 100% RDF for base crop + foliar spray of 2% DAP +1% KCl (N_4), followed by (N_5) 100% RDF for base crop + 50%RDF for intercrops+ foliar spray of 2% DAP +1%KCl, (47.98, 58.94 and47.41, 56.49 mg g^{-1} FW) in both pre flowering and flowering stage. Application of PPFM @ 500 ml ha^{-1} (D2) foliar spray recorded higher proline content and was followed by foliar spray of 500 ppm cycocel (CCC) (D3) in both the stages (45.44, 51.55 and 43.59, 49.05mg g^{-1} FW). The interaction effect between different cropping system, nutrient and stress management exhibited siginificant variations. The higher amount of proline was noticed in Pigeon pea + foxtail millet + Groundnut (1:2:1) (M_2) + (N_4) 100% RDF for base crop + foliar spray of 2% DAP +1% KCl + foliar spray of PPFM @500 ml ha^{-1} ($M_2N_4D_2$) registered higher proline content (52.85 and 64.85 mg g^{-1} FW) for both stage of crops.

The accumulation of proline is tightly controlled by genes and cDNA encoding osmolyte biosynthesis and is only achieved when the rate of synthesis prevails over that degradation, probably because too much proline is toxic to plant cell. As proline has hydrophilic property, it might replace water molecules around nucleic acid, protein and membranes during water shortages. It might also prevent interaction between destabilize ions andcellular components by replacing the water molecules around these components, thereby protecting against destabilization during drought, Similar results were observed by Yokota *et al.* 2006, Priyanka *et al,* 2010 and Turan *et al.*, 2007. Foliar spray of potassium induces proline synthesis and this proline might have served as a compatible solute.

High levels of proline synthesis during stress may maintain NAD (P) + NAD(P)H ratios at values compatible with metabolism under normal conditions. This increased ratio mediated by proline biosynthesis is likely to enhance activity of the oxidative pentose phosphate pathway. This would provide precursors metabolite production during stress as well as nucleotide synthesis, accompanying the accelerated rate of cell division upon relief from stress when oxidation of proline is likely to provide an important energy source for ADP phosphorylation. It is advantageous for the plants to keep the cells in turgid state as proline is highly soluble and stable to acid hydrolysis. The similar result was observed by Gadallah 1999.

Small Millets (Figure 1 & 2)

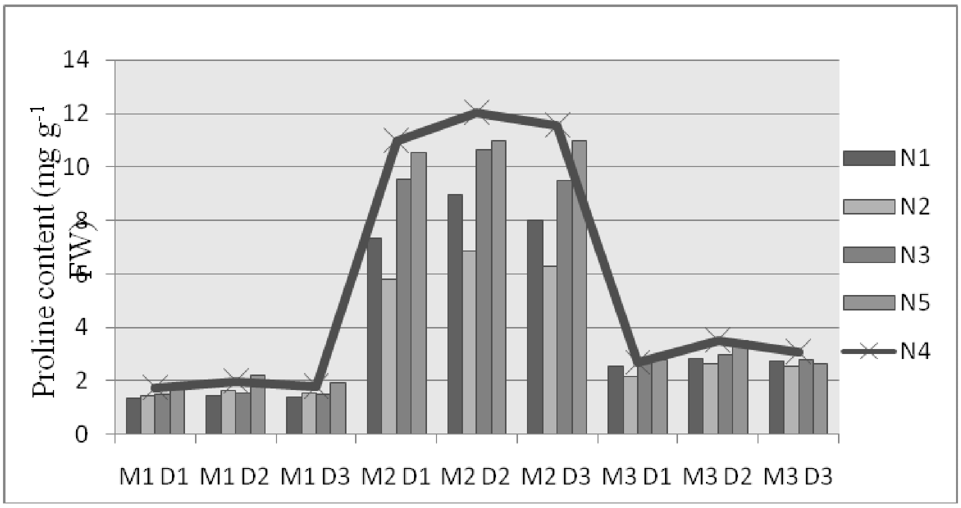

Figure 1: Accumulation of Proline in Small Millets (mg g^{-1} FW) at Vegetative Stage under Different Nutrient and stress Management in Pigeon Pea Based Multi-Tier Cropping System under Rainfed Condition During *rabi* 2015-2016

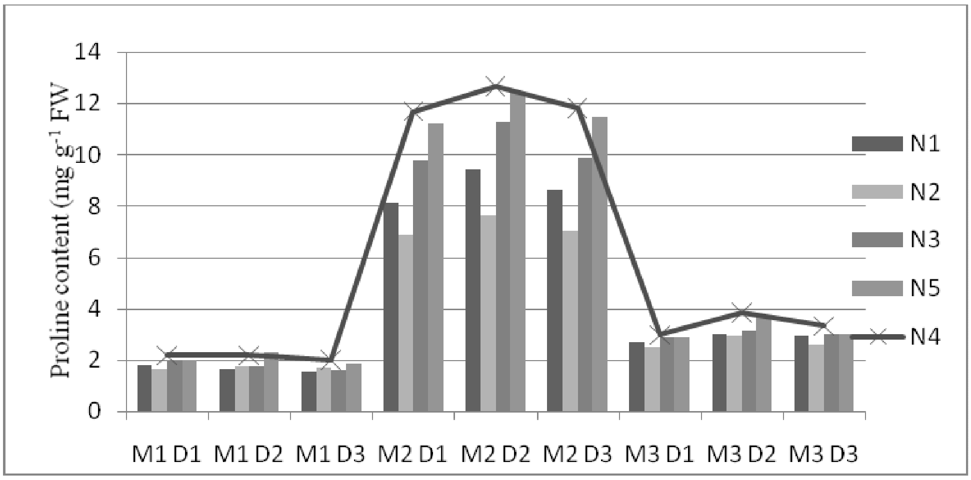

Figure 2: Accumulation of Proline in Small Millets (mg g^{-1} FW) at Flowering Stage under Different Nutrient and Stress Management in Pigeon Pea Based Multi-Tier Cropping System under Rainfed Condition During *rabi* 2015-2016

Cropping system, different nutrient and stress management techniques significantly influenced the proline accumulation of small millets at all the crop growth stages. The highest proline content was recorded (M_2) Pigeon pea + foxtail millet + Groundnut (1:2:1) followed by (M_3) Pigeon pea + kodo millet + Groundnut (1:2:1) (9.34, 9.98 and 2.80, 3.03 mg g^{-1} FW) both pre flowering and flowering stage. Among the nutrient management, application 100% RDF for base crop + foliar spray of 2% DAP +1% KCl (N_4) recorded highest proline content (5.49 and 5.86 mg g^{-1} FW) followed by (N_5) 100% RDF for base crop + 50%RDF for intercrops+ foliar spray of 2% DAP +1%KCl,(5.27 and 5.65 mg g^{-1} FW) in both the stage of crop. With regard to stress management (D_2) foliar spray of PPFM @500 ml ha^{-1} was recorded highest proline content followed by (D_3) foliar spray of 500 ppm cycocel (CCC) in both the stages (4.92, 5.30 and 4.54, 4.82 mg g^{-1} FW). The interaction effect between cropping system with different nutrient and stress management techniques were found to be non significant.

The increase in the concentrations of proline in minor millets was found to be remarkable during drought stress. These results suggest that the production of these osmotic adjustments is a common response of plants under drought conditions. Osmotic adjustment through the accumulation of cellular solutes, such as proline, has been suggested as one of the possible means for overcoming osmotic stress caused by loss of water (Caballero *et al.*, 2005). Proline is a non-protein amino acid that forms in most tissues subjected to water stress and together with sugar, it is readily metabolized upon recovery from drought (Singh *et al.*, 2000). In addition to acting as an osmo-protectant, proline also serves as a sink for energy to regulate redox potentials, as a hydroxyl radical scavenger (Sharma and Dietz, 2006), as a solute that protects macro-molecules against denaturation and as a means of reducing acidity in the cell (Kishor *et al.*, 2005).

In addition to acting as an osmo-protectant, proline also serves as a sink for energy to regulate redox potentials, as a hydroxyl radical scavenger (Sharma and Dietz, 2006), as a solute that protects macro-molecules against denaturation and as a means of reducing acidity in the cell (Kishor *et al.*, 2005). However, Vendruscolo *et al.*, 2007 stated that proline might confer drought tolerance to wheat plants by increasing the antioxidant system rather than as an osmotic adjustment.

Peanut (Table 3 & 4)

Among the cropping system (M_2) Pigeon pea + foxtail millet + Groundnut (1:2:1) recorded higher proline content (2.39, 2.69 mg g^{-1} FW) followed by (M_1) Pigeon pea + barnyard millet + Groundnut (1:2:1) (2.21, 2.51 mg g^{-1} FW) in both pre flowering and vegetative stage of the crop. With regard to other agronomic management, application of 100% RDF for base crop + foliar spray of 2% DAP (N_4) +1% KCl was registered significantly higher proline content (2.79, 3.09, mg g^{-1} FW) followed by (N_5) 100% RDF for base crop + 50%RDF for intercrops+ foliar spray of 2% DAP +1%KCl (2.64, 2.94 mg g^{-1} FW) in both stage of crop. Significant effect was noticed in various stress management practices. Foliar spray of PPFM @500 ml ha^{-1} (D_2) recorded highest proline content (2.43, 2.73 mg g^{-1} FW) followed by (D_3) foliar spray of 500 ppm cycocel (CCC) (2.19, 2.49 mg g^{-1} FW) in pre flowering and flowering stages.

The interaction effect between cropping system with different nutrient and stress management techniques was found to be significant. Pigeon pea + foxtail millet + Groundnut (1:2:1) (M_2) + (N_4) 100% RDF for base crop + foliar spray of 2% DAP +1% KCl + foliar spray of PPFM @500 ml ha^{-1} ($M_2N_4D_2$) registered higher proline content (3.15 and 3.45 mg g^{-1} FW) for both stage of crops.

The accumulation of free proline in stressed plants has been found to be an adaptive mechanism for drought tolerance and a positive correlation between magnitude of free proline accumulation and drought tolerance has been considered as an index for determining drought tolerance potential of cultivars. The similar result was observed by Upadhaya, *et al.*, 2008. The accumulation of proline in dehydrated plants is caused both activation of biosynthesis of proline and by inactivation of the degradation of proline. P5C reductase, a key enzyme catalyzes the final step in the biosynthetic pathway leading from glutamic acid to proline. An increase, in activity of P5C reductase correlated with proline accumulation under water stress has been reported (Ramanjulu and Sudhakar, 2000). The experimental evidence in this study also suggests that increase in the P5C reductase activity could be one of the reasons for the higher accumulation of proline at all stress regimes. Another important factor that contributed to proline accumulation during stress is the degradation of proline. Proline is oxidized to glutamine in the mitochondria by sequential action of proline oxidase and proline dehydrogenase. In the present study, we observed inhibition of both proline oxidizing enzyme in the leaves of groundnut during water stress. These results are in agreement with earlier reports (Ramanjulu and Sudhakar 2000).

CONCLUSIONS

Thus based on results of the present investigation clearly demonstrate that Pigeon pea + foxtail millet + Groundnut (1:2:1) + 100% RDF for base crop + foliar spray of 2% DAP +1% KCl + foliar spray of PPFM @ 500 ml ha^{-1} multi-tier cropping system with different nutrient and stress management practice was productive in terms of moisture stress under rainfed condition

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Table 1: Accumulation of Proline in Pigeonpea (mg g^{-1} FW) at Vegetative Stage under Different Nutrient and Stress Management in Pigeon Pea Based Multi-tier Cropping System under Rainfed Condition During *rabi* 2015-2016

Treatments	N_1	N_2	N_3	N_4	N_5	Mean
M_1	40.73	35.74	43.09	47.65	45.15	**42.47**
M_2	43.3	39.11	43.72	49.11	49.51	**44.95**
M_3	41.33	38.73	42.66	47.19	47.58	**43.5**
Mean	**41.79**	**37.86**	**43.16**	**47.98**	**47.41**	
D_1	40.65	35.93	42.6	44.68	45.72	**41.92**
D_2	42.79	39.63	43.81	51.52	49.43	**45.44**
D_3	41.92	38.03	43.05	47.75	47.1	**43.57**
Mean	**41.79**	**37.86**	**43.16**	**47.98**	**47.41**	
$M_1 D_1$	39.35	33.85	42.56	44.55	44.05	**40.87**
$M_1 D_2$	41.85	37.5	43.85	50.85	46.55	**44.12**
$M_1 D_3$	41	35.88	42.85	47.56	44.85	**42.43**
$M_2 D_1$	42.05	37.5	43.17	45.65	47.85	**43.24**
$M_2 D_2$	44.18	40.88	44.05	52.85	51.89	**46.77**
$M_2 D_3$	43.68	38.95	43.95	48.84	48.8	**44.84**
$M_3 D_1$	40.56	36.45	42.08	43.85	45.25	**41.64**
$M_3 D_2$	42.35	40.5	43.54	50.86	49.85	**45.42**
$M_3 D_3$	41.09	39.25	42.35	46.85	47.64	**43.44**
Mean	**41.79**	**37.86**	**43.16**	**47.98**	**47.41**	
	SE.d			**CD(p=0.05)**		
M	NS			NS		
N	1.11			2.42		
D	0.61			1.25		
M x N x D	2.6			5.46		

Table 2: Accumulation of Proline in Pigeonpea (mg g^{-1} FW) at Flowering Stage under Different Nutrient and Stress Management in Pigeon Pea Based Multi-tier Cropping System under Rainfed Condition During *rabi* 2015-2016

Treatments	N_1	N_2	N_3	N_4	N_5	Mean
M_1	41.81	43.43	43.92	54.92	51.15	**47.04**
M_2	45.22	44.27	45.92	62.89	60.38	**51.74**
M_3	45.07	42.83	45.65	59.02	57.93	**50.10**
Mean	**44.03**	**43.51**	**45.16**	**58.94**	**56.49**	
D_1	43.48	42.85	43.25	56.45	55.35	**48.28**
D_2	44.37	45.70	47.38	61.65	58.65	**51.55**
D_3	44.24	41.98	44.85	58.73	55.47	**49.05**
Mean	**44.03**	**43.51**	**45.16**	**58.94**	**56.49**	
$M_1 D_1$	44.85	40.69	40.85	52.25	49.75	**45.68**
$M_1 D_2$	39.54	46.35	46.35	57.95	53.65	**48.77**
$M_1 D_3$	41.03	43.25	44.55	54.56	50.05	**46.69**
$M_2 D_1$	41.35	44.36	43.75	61.25	59.55	**50.05**
$M_2 D_2$	47.55	45.89	48.35	64.85	62.35	**53.80**
$M_2 D_3$	46.75	42.55	45.65	62.58	59.25	**51.36**
$M_3 D_1$	44.23	43.50	45.15	55.85	56.75	**49.1**
$M_3 D_2$	46.02	44.85	47.45	62.15	59.95	**52.08**
$M_3 D_3$	44.95	40.15	44.35	59.05	57.1	**49.12**
Mean	**44.03**	**43.51**	**45.16**	**58.94**	**56.49**	

	SE.d	CD(p=0.05)
M	NS	NS
N	1.23	2.69
D	1.23	2.52
M x N x D	4.36	9.02

Table 3: Accumulation of Proline in Peanut (mg g^{-1} FW) at Vegetative Stage under Different Nutrient and Stress Management in Pigeon Pea Based Multi-Tier Cropping System under Rainfed Condition During *rabi* 2015-2016

Treatments	N_1	N_2	N_3	N_4	N_5	Mean
M_1	1.76	1.68	2.30	2.73	2.59	**2.21**
M_2	1.89	1.94	2.40	2.92	2.78	**2.39**
M_3	1.82	1.70	2.15	2.73	2.55	**2.19**
Mean	**1.82**	**1.77**	**2.28**	**2.79**	**2.64**	
D_1	1.71	1.76	2.19	2.65	2.52	**2.16**
D_2	1.97	1.90	2.47	3.00	2.81	**2.43**
D_3	1.78	1.66	2.19	2.73	2.60	**2.19**
Mean	**1.82**	**1.77**	**2.28**	**2.79**	**2.64**	
M_1 D_1	1.60	1.73	2.17	2.60	2.45	**2.11**
M_1 D_2	1.85	1.78	2.50	2.95	2.78	**2.37**
M_1 D_3	1.83	1.53	2.23	2.63	2.55	**2.15**
M_2 D_1	1.83	2.00	2.30	2.75	2.70	**2.32**
M_2 D_2	2.06	2.05	2.60	3.15	2.75	**2.52**
M_2 D_3	1.78	1.78	2.30	2.85	2.90	**2.32**
M_3 D_1	1.70	1.55	2.10	2.60	2.40	**2.07**
M_3 D_2	2.00	1.88	2.31	2.90	2.90	**2.40**
M_3 D_3	1.75	1.68	2.05	2.70	2.35	**2.11**
Mean	**1.82**	**1.77**	**2.28**	**2.79**	2.59	
	SE.d			**CD(p=0.05)**		
M	0.06			0.11		
N	0.05			0.09		
D	0.06			0.13		
M x N x D	0.08			0.15		

Table 4: Accumulation of Proline in Peanut (mg g^{-1} FW) at Flowering Stage under Different Nutrient and Stress Management in Pigeon Pea Based Multi-Tier Cropping System under Rainfed Condition During *rabi* 2015-2016

Treatments	N_1	N_2	N_3	N_4	N_5	Mean
M_1	2.06	1.98	2.60	3.03	2.89	**2.51**
M_2	2.19	2.24	2.70	3.22	3.08	**2.69**
M_3	2.12	2.00	2.45	3.03	2.85	**2.49**
Mean	**2.12**	**2.07**	**2.58**	**3.09**	**2.94**	
D_1	2.01	2.06	2.49	2.95	2.82	**2.46**
D_2	2.27	2.20	2.77	3.30	3.11	**2.73**
D_3	2.08	1.96	2.49	3.03	2.90	**2.49**
Mean	**2.12**	**2.07**	**2.58**	**3.09**	**2.94**	
M_1 D_1	1.90	2.03	2.47	2.90	2.75	**2.41**
M_1 D_2	2.15	2.08	2.80	3.25	3.08	**2.67**
M_1 D_3	2.13	1.83	2.53	2.93	2.85	**2.45**
M_2 D_1	2.13	2.30	2.60	3.05	3.00	**2.62**
M_2 D_2	2.36	2.35	2.90	3.45	3.05	**2.82**
M_2 D_3	2.08	2.08	2.60	3.15	3.20	**2.62**
M_3 D_1	2.00	1.85	2.40	2.90	2.70	**2.37**

Table 4

M_3 D_2	2.30	2.18	2.61	3.20	3.20	**2.70**
M_3 D_3	2.05	1.98	2.35	3.00	2.65	**2.41**
Mean	**2.12**	**2.07**	**2.58**	**3.09**	**2.94**	
	SE.d			**CD(p=0.05)**		
M	0.07			0.14		
N	0.06			0.11		
D	0.08			0.15		
M x N x D	0.09			0.17		