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Effect of Triacontanol on Seed Germination, Seedling Growth and Antioxidant Enzyme in Rice Under Poly ethylene glycol Induced Drought Stress

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ABSTRACT

Investigations were carried out to document the effect of bioregulator triacontanol with two concentrations (5, 10 µg) on seed germination seedling growth in rice during 2011. The results revealed that seed germination was increased with treatment of triacontanol over all the treatments and improved the growth of polyethylene glycol -20 % (mw-6000) + Triacontanol treated seeds compared with polyethylene glycol -20%. Length, fresh and dry weights were also found to increase with triacontanol treatment. Catalase, Peroxidase and Superoxide dismutase activities were also discriminated by the treatment under polyethylene glycol induced drought conditions.

Key words : Antioxidant enzymes, Germination, Growth, Polyethylene glycol induced drought, Rice triacontanol.

Rice (*Oryza sativa* L.) is an important food cereal belongs to Poaceae family. The share of the Asian farmers is 92% in world's total rice production (Kondamudi et al., 2011). Rice as a paddy field crop is particularly susceptible to water stress (Tao et al., 2006), the variety IR64 is highly susceptible to drought stress (Lafitte et al., 2007).

Drought is the most important limiting factor for crop production and it is becoming severe problem in many regions of the world (Passioura, 2007). According to statistics, the percentage of drought affected land areas more than doubled from the 1970s to the early 2000s in the world (Isendahl et al., 2006). With increasing population and global climate change makes the situation more serious (Hongbo et al., 2005). It is estimated that 50% of the world rice production is affected more or less by drought (Bouman et al., 2005). Drought stress was found to reduce dry weight, root traits, water potential, photosynthetic parameters (Chen et al., 2010).

Triacontanol is a saturated long-chain primary alcohol that is known to have a growth enhancing activity. Earlier research proved that improving growth and yield of several plants with application of triacontanol (Borowski *et al.,* 2000). Effect of triacontanol on soybean plants under salt stress was proved in restoring the normal metabolic process (Radhakrishnan and Ranjitha Kumari 2008). Triacontanol application increased the yield in groundnut plants (Kabadagi and settee 2010). Shagufta et al., (2010) reported a marginal increase was found in photosynthetic rate in wheat plants under salt stress by the application of triacontanol. Triacontanol induced seed germination, seedling growth was found in mungbean by Puspendu and Bera (2007).

The present studies were carried out to investigate the influence of triacontanol on seed germination, seedling growth and antioxidant system in rice seedlings under drought stress.

MATERIAL AND METHODS

Laboratory experiment was conducted at Directorate of Rice Research Institute, Hyderabad, during Scptember to December 2011.

For drought stress initiation, one of the most popular approaches is to use high molecular weight osmotic substances, like polyethylene glycol (Landjeva *et al*., 2008). In current study, polyethylene glycol was used for drought stress induction in rice seedlings.

Seed germination

IR-64 seeds were surface sterilised with dilute sodium hypochlorite solution (with 0.5% v/v) and washed thoroughly with several changes of sterile distilled water. Twenty seeds were placed in

petriplate. Four ml of either of the test solution or distilled water (Control)/20% polyethylene glycol solution supplemented with 5,10 µg of triacontanol was added to different petriplates. The seeds were allowed to germinate in dark at 20±10 ^{O}C. Every 12h number of seeds germinated was recorded in each plate. 2 ml more test solution was added every alternate day. On 7th day, the growth of the seedlings was recorded in terms of plumule and radicle weight and estimated antioxidant enzymes of Catalase, Peroxidase, Superoxide dismutase and the activity is expressed in mg g^{-1} fresh weight.

Catalase (EC 1.11.1.6)

Catalase activity was assayed by the method of Barber (1980). Enzyme extract was added to hydrogen peroxide and phosphate buffer (pH 7.0). The reaction was stopped by adding concentrated sulphuric acid, and the residual hydrogen peroxide was titrated against $KMnO_4$ until a faint purple colour persisted for atleast 15 seconds.

Peroxidase (EC 1.11.1.7*)*

Peroxidase activity was assayed adopting the method of Kar and Mishra (1976). Phosphate buffer (pH 7.0), pyrogallol and hydrogen peroxide were added to enzyme extract. After incubation, the reaction was stopped by adding sulphuric acid. The amount of purpurogallin formed was estimated by measuring the absorbance at 420nm.

Superoxide dismutase (EC 1.15. 1.1)

Superoxide dismutase activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium adopting the method of Beauchamp and Fridovich (1971). The reaction mixture contained phosphate buffer (pH 7.8), Methionine, NBT, EDTA, enzyme extract and riboflavin, which was added at the end. After mixing the contents, test tubes were shaken and placed 30 cm below light source. The absorbance was measured at 540nm.

Statistical analysis:

The data represents the mean values of 5 replicates and 3 replicate for Catalase, Peroxidase and Superoxide dismutase. The data were analyzed by identifying least significant difference (LSD) and one-way ANOVA, followed by Multiple Comparisons with Statistix-8.1 software. The differences were considered significant if P was at least d" 0.05. The mean values have been compared and lower case alphabets are used in the tables and figures to highlight the significant differences between the treatments.

RESULTS AND DISCUSSION

Growth parameters markedly decreased due to polyethylene glycol treatment in rice (Table-1).However application of Triacontanol significantly increased germination percentage than the other treatments with increased concentration. Polyethylene glycol + Triacontanol also showed increase over polyethylene glycol induced drought stress respectively (Table-1). The results clearly revealed that the difference between drought stress and hormonal treatment to polyethylene glycol induced drought stressed seeds (Triacontanol -5 µg + polyethylene glycol 20%, triacontanol -10 µg+ polyethylene glycol 20%) in terms of seed

Table-1. Effect of triacontanol on seed germination under poly ethylene glycol induced drought stress in rice.

	Seed germination (%)			
Treatments	12h	24h	36h	48h
control	8.3 a	30 a	90 ab	95 abc
triacontanol -5 µg	11.6 a	31.6 a	95 a	96.6 ab
triacontanol -10 µg	13.3 a	33.3 a	96.6 a	98.3 a
Poly ethylene glycol-20%	0 b	0 b	18.3 c	31.6 d
triacontanol -5 µg + Poly ethylene glycol-20%	0 b	3.3 b	80 b	85 bc
triacontanol -10 µg + Poly ethylene glycol-20%	0 b	11.6 b	81.6 b	86.6 bc
LSD	1.62	3.02	2.51	2.09

Plate-1. Effect of triacontanol on rice seedling growth under poly ethylene glycol induced drought stress.

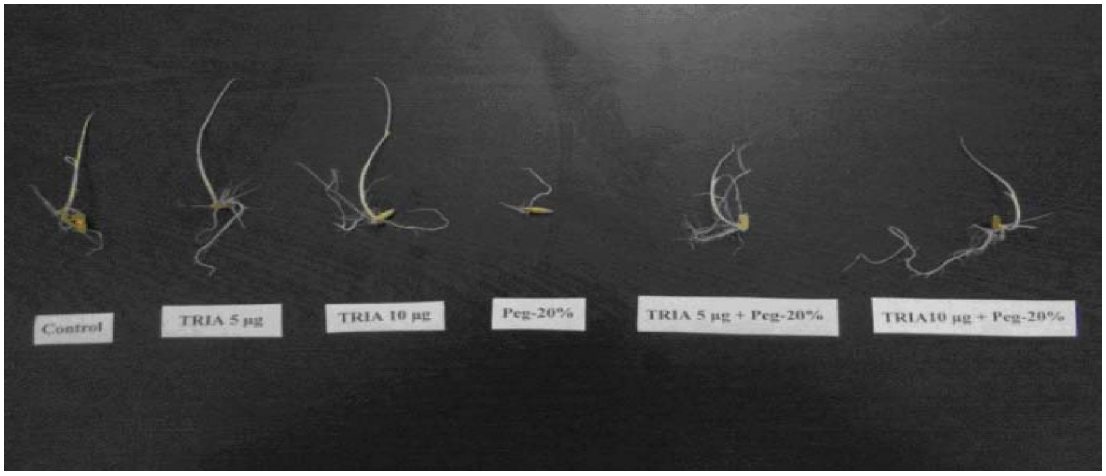


Table-2. Influence of triacontanol on seedling growth under poly ethylene glycol induced drought stress in rice.

	Length (cm)		Fresh weight (µg)		Dry weight (µg)	
Treatment	Plumule	Radicle	Plumule	Radicle	Plumule	Radicle
control	3.44 c	4.54 b	145.8 d	57.8 c	23 c	9.8 c
triacontanol -5 µg	4.54 b	4.74 b	285.6 b	66.8 abc	33 b	13.8 c
triacontanol -10 µg	5.64 a	5.38 b	375.2 a	75.6 a	45 a	26 a
Poly ethylene glycol-20	1.16 d	0.22 c	31.0 e	1.8 d	3.8 d	0.2 d
triacontanol -5 µg + Poly ethylene glycol-20	3.14 c	5.06 b	235.6 c	60.6 bc	25.6 c	19.2 b
triacontanol -10 µg + Poly ethylene glycol-20	4.24 b	6.96 a	313 b	72.4 ab	37.6 b	22.6 ab
LSD	0.48	1.05	37.00	12.62	6.93	5.19

germination percentage at 48h was 31.6%, 85%, 86.6% respectively.

Rice seedling growth was effected by polyethylene glycol concentration. The physical process of water uptake leads to the activation of metabolic processes as the dormancy of the seed is broken following hydration. Elevated polyethylene glycol slows down water uptake by seeds, thereby inhibiting their germination shoot and root elongation. Polyethylene glycol solutions of increasing concentration were probably caused by the decrease in water potential gradient between the seeds and their surrounding media (Bewley and Black, 1994). Polyethylene glycol may readily cross the cell membrane into the cytoplasm of the cells unless an active metabolic pump prevents accumulation of the ions.

Earlier results of Swamy and Rao (2005) proved application of Brassinosteroids decreased the lead toxicity in fenugreak seeds at germination level. Salicylic acid eliminated the adverse effects of cold stress in bean seeds by Gharib and Hegazi (2010). Similarly a lower concentration of triacontanol increased seed germination of cow pea under water stress by Tilottama et al., (2010).

Seedling growth in terms of length, fresh and dry weights (plumule and radical) were found increased with triacontanol treatment (Plate-1). A remarkable inhibition was found in polyethylene glycol induced drought seedling growth and it was

removed by triacontanol treatment (Table-2). Polyethylene glycol + Triacontanol treated seeds proved acute difference over poly ethylene glycol induced drought seedlings.

Similarly Cavusoglu et al., (2007) reported barley seedling growth was affected by the application of triacontanol under saline conditions. Application of triacontanol was increased the wheat plant growth under salinity by Bagdi and Afria (2008). Supplementation of triacontanol had a significant effect on yield attributes of mustard plant by Fozia et al., (2006).

To understand the protective action of antioxidants against polyethylene glycol induced drought stress, rice seedlings were treated with triacontanol followed by measurement of the level of antioxidant activity (Figure 1, 2 and 3). Increasing triacontanol concentration significantly increased enzyme activity under stress conditions.

The excessive generation of Reactive oxygen species (ROS) in plants under drought stress such as singlet oxygen (1/2 O_2), superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH) were scavenged by the antioxidant enzymes like Catalase, Peroxidase, Superoxide dismutase etc. Catalase is one of the highest of all enzymes which converts millions of molecules of hydrogen peroxide to water and oxygen in one second.

Catalase activity was increased in triacontanol as the concentration augmented over control, polyethylene glycol + triacontanol significantly increased over polyethylene glycol induced drought treated seeds (Fig-1). Previous

Fig-1. Influence of triacontanol on catalase activity in rice seedlings under poly ethylene glycol induced drought stress

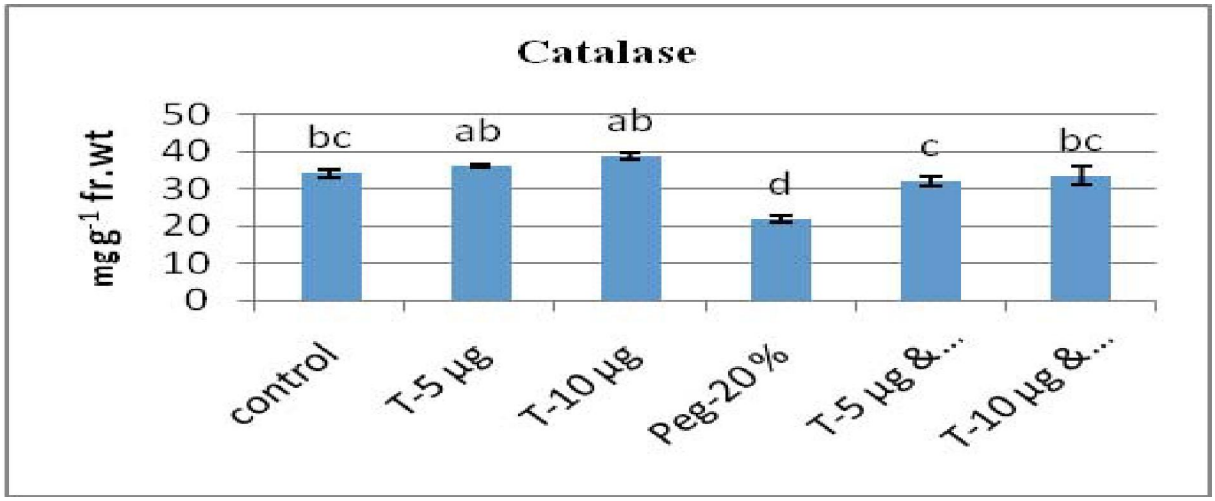


Fig-2. Influence of triacontanol on peroxidase activity in rice seedlings under poly ethylene glycol induced drought stress

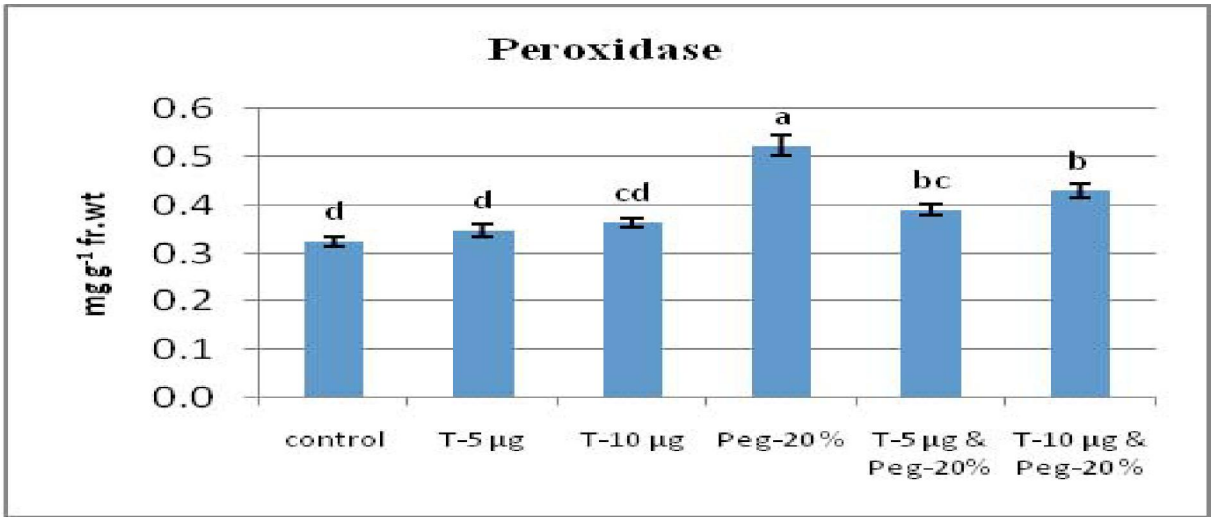
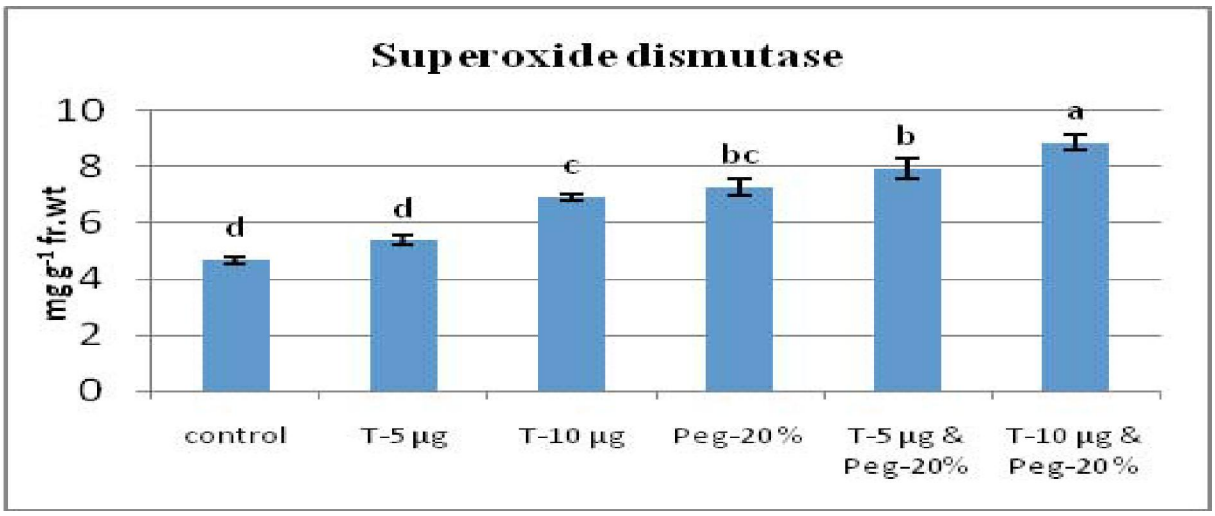


Fig-3. Influence of triacontanol on superoxide dismutase activity in rice seedlings under poly ethylene glycol induced drought stress



studies showed that application of 24-epibrassinoslide increased catalase activity in bean plants under cadmium stress by Mostafa (2011).

The highest Peroxidase activity was found in polyethylene glycol induced drought seedlings than the other treatments. Gradually enhanced the Peroxidase activity in triacontanol and polyethylene glycol + triacontanol treated seedlings with a concentration increases over control (Fig-2). Peroxidase is also known to play significant role in oxidative stress conditions. The mode of action of Peroxidase on H_2O_2 substrate differs from Catalase in that Peroxidase liberates free radicals rather than oxygen.

The higher Superoxide dismutase activity was noticed in polyethylene glycol + triacontanol treatment as concentration increases over all the treatments. Only triacontanol treatments were found increased over control (Fig-3). Superoxide dismutase an important defense enzymes catalyze the dismutation and Chen et al., (1997) found the application of homobrassinolide induced peroxidase and superoxide dismutase activities in rice.

The results obtained in the present study clearly demonstrated the ameliorative effect of triacontanol on polyethylene glycol induced drought stress. The increased triacontanol concentration also improved the protection against polyethylene glycol induced drought stress damage to the rice seedlings. Hence, it can be concluded that the supplementation of triacontanol proved to be beneficial for rice seedlings under drought stress conditions.

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LITERATURE CITED

Bagdi D L, and B S Afria 2008 Alleviation of salinity effects using plant growth regulators in wheat. *Indian Journal of Plant Physiology*, 2008. 13(3): 272-277.

Barber J M 1980 Catalase and peroxidase in primary leaves during development and senescence. *Zeitschrift fur Pflanzen Physiologie*, 97: 135–144.

Beauchamp C, and I Fridovich 1971 Superoxide dismutase: improved assay and an assay applicable to acrylamide gels. *Analytical Biochemistry*, 44: 276–287.

Bewley DJ, and M Black 1994 *Seeds: Physiology of development and germination. New York: Plenum Press.*

Borowski, E Z K Blamowski and W Michalek 2000 Effects of Tomatex/Triacontanol on chlorophyll fluorescence and tomato (*Lycopersicon esculentum* Mill.) yields. *Acta Physiologia Plantarum,* 22: 271-274.

Bouman B A M, S Peng, A R Castaòeda and R M Visperas 2005 Yield and water use of irrigated tropical aerobic rice systems. *Agricultural Water Management*, 74(2): 87-105.

Cavusoglu K, S Klc and K Kabar 2007 Effects of triacontanol pretreatment on seed germination, seedling growth and leaf anatomy under saline (NaCl) conditions. *Suleyman Demirel Universitesi Fen Edebiyat Fakultesi Fen Dergisi*, 2(2): 136-145.

Chen S N, J N Li, H L You, H J Zhu, Z B Qin, G M Hong and Y G Shen 1997 The effect of a compound inducing cold resistance and homobrassinolide on the chilling resistance of plateau rice. *Acta Botanica Yunnanica,* **19:** 184–190.

Fozia-Rasid, Dinesh-Sah and Shamsul-Khaliq 2006 Response of triacontanol and nitrogen levels on growth, yield, quality and nutrient uptake of mustard (Brassica juncea L.). *Environment and Ecology*, 24S(Special 2): 281-284.

Gharib F A and A Z Hegazi 2010 Salicylic Acid Ameliorates Germination, Seedling Growth, Phytohormone and Enzymes Activity in Bean (*Phaseolus vulgaris* L.) under Cold Stress. *Journal of American Science,* 2010. 6 675-683.

Hongbo S, L Zongsuo and S Mingan 2005 Changes of anti-oxidative enzymes and MDA content under soil water deficits among 10 wheat (*Triticum aestivum* L.) genotypes at maturation stage. Colloids and Surfaces B: *Biointerfaces*, 45(1): 7-13.

Isendahl N and G Schmidt 2006 Drought in the mediterranean-WWF policy proposals. In A, edited by W. Report. *Madrid.*

Kabadagi C B and R A Setty 2010 Growth characters and yield of groundnut genotypes as influenced by levels of NPK and growth regulators. *Research on Crops*, 2010. 11(3): 697-700.

Kar M and D Mishra 1976 Catalase, peroxidase and polyphenol oxidase activities during rice leaf senescence. *Plant Physiology*. 57: 315–331.

Kondamudi R, K N Swamy, D V N Chakravarthy, V Vishnuprasanth, Y V Rao, P R Rao , N Sarla, D Subramanyam and S R Voleti 2012 "Heat stress in rice – physiological mechanisms and adaptation strategies". *Crop stress and its management: Perspectives and Strategies – Springer*, 193-224.

Lafitte1 H R, G Yongsheng, S Yan and Z K Li1 2007 Whole plant responses, key processes, and adaptation to drought stress: the case of rice. *Journal of Experimental Botany*, 58, 169–175.

Landjeva S, K Neumann, U Lohwasser and Borner 2008 Molecular mapping of genomic regions associated with wheat seedling growth under osmotic stress. *Biologia Plantarum,* 52: 259-266.

Mostafa M Rady 2011 Effect of 24-epibrassinolide on growth, yield, antioxidant system and cadmium content of bean (*Phaseolus vulgaris* L.) plants under salinity and cadmium stress. *Scientia Horticulturae*, 129: 232-237.

Passioura J B 2007 The drought environment: physical, biological and agricultural perspectives. *Journal of Experimental Botany*. 58(2): 113-117.

Puspendu D and A K Bera 2007 Effect of trinol on seed germination and seedling growth of mungbean. *Legume Research*, 2007 30(2): 151-153.

Radha Krishnan R and B. D. Ranjitha Kumari 2008 Effect of n-triacontanol on the growth of salt stressed soybean plants. *Journal of Bioscience*, 19: 53–62.

Shagufta Perveen, Muhammad Shahbaz and Muhammad Ashraf 2010 Regulation in gas exchange and quantum yield of photosystem II (PSII) in salt-stressed and non-stressed wheat plants raised from seed treated with triacontanol. *Pakistan Journal of Botany*, 42(5): 3073-3081.

Swamy K N and S S R Rao 2005 "Brassinosteroids reduced the inhibitory effect of lead toxicity on seed germination and seedling growth of *Trigonella foenum – graceum* L." *Bioinfolets*, 1: 32-34.

Tao H, H Brueck, K Dittert, C Kreye, S Lin and B Sattelmacher 2006 Growth and yield formation for rice (*Oryza sativa* L.) in the water saving ground cover rice production system (GCRPS). *Field Crops Research*, 95: 1-12.

Tilottama, N Raghava and R P Raghava 2010 Effect of miraculan of seed germination parameters in cowpea under water stress. *Biosciences,-Biotechnology-Research-Asia*, 7(1): 353-358.

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