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Evaluation of the growth parameters AGR, RGR and NAR of soybean [*Glycine max* (L.) Merr.] under Cd (II) stress

VASUDEVAN MURALI KRISHNAN AND V.N. SATAKOPPAN

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SUMMARY

Heavy metals are one of the major toxic pollutants resulted due to various industrial developments and anthropogenic activities, which should be considered, for their innumerable negative effects on ecology, evolution, health, nutrition and environment. Anthropogenic activities and industrial activities have polluted the soil and water bodies extensively. Plants posses various types of cellular mechanisms to handle and regulate the levels of metal ions inside the cell and to minimize the heavy metal toxicity that could result from the exposure to heavy metals contaminated environment. Among the heavy metals, Cd is of special concern because of its potential toxicological profile even at lower concentration. This study aims to evaluate the various growth parameters such as AGR, RGR and NAR of Co-2 variety soybean under heavy metal Cadmium stress (0, 5, 10, 15, 20 and 25 ppm). The observation was done on 20th day and 40th day of its growth and the results were compared and evaluated. This study demonstrated the need for understanding and evaluation of the effects of effluents or wastewater containing cadmium on soybean which may help to improve the production of soybean to meet the increasing global demands and for development of effective treatment methods for reuse of waste water for agriculture.

Key words : Soybean, Cadmium, AGR, RGR, NAR.

The increasing contamination and consequential accumulation of heavy metals in the soil by anthropogenic activities, in particular the disposal of sewage sludge can be very serious, if the crops are grown and cultivated in such polluted environment (Wagner, 1993). The current worldwide mine production of various heavy metals such as Cu, Cd, Pb, and Hg is very high (Pinto *et al.*, 2004). Metal toxicity and its effect on the growth and metabolism in higher plants is a subject that has a wide economical and ecological interest and also have been widely reviewed and gaining importance on several occasions over the last few decades (Brown and Jones, 1975; Foy *et al.*, 1978; Ernst *et al.*, 1992; Das *et al*., 1997; Sanitá di Toppi and Gabbrielli, 1999; Hall, 2002; Clemens *et al.*, 2002). Another interesting reason for this is out of the ninety naturally occurring elements fifty-three are heavy metals as reported by Weast (1984). Few metals are found to be essential for growth of plants, while few are found to be toxic for the growth of plants. Some of the heavy metals do not interact with the plant metabolism directly but decreases the level of soil microbes, affecting the growth of the plants (Niess, 1999).

The background cadmium (Cd) level in agricultural soils is found to be less than 1mg.kg^{-1} (Adriano, 2001). However, higher values until 30 ppm have been observed in many agricultural and contaminated soils due to long-term use of phosphate fertilizers, release of effluents to water bodies and agricultural lands, contamination of ground water levels with cadmium and sewage sludge application (Chaney, 1980). Increased Cd levels were also found in the surface soils near the metal processing industry. High mobility of this metal in soil-plant system allows its easy entering into food network (Ryan *et al.*, 1982) where it may provoke both human diseases (Nogawa *et al.*, 1987) and well-known toxicity effects on animals, microorganisms and plants. Increasing international concern about the risks associated with long-term consumption of crops contaminated with Cd has led the international food standards organization, Codex Alimentarius Commission, to propose a 0.1mg Cd.kg^{-1} dry weight limit for cereals, pulses and legumes (Harris and Taylor, 2001).

Soybean is an important legume crop, which is an excellent source of protein, which is also referred to as poor man's meat (Tandale and Ubale, 2007). The low productivity of soybean is observed in India, comparing to other countries. In addition, the water scarcity and pollution of the agricultural land has become a big threat for agriculture in India and worldwide. Hence, new agricultural practices or new irrigational techniques are to be developed and practiced in order to meet the current demand for soybean, as a food source and source of non-

Correspondence to:
VASUDEVAN MURALI KRISHNAN, Department of Biochemistry, P.S.G. College of Arts and Science, COIMBATORE (T.N.) INDIA
Authors' affiliations:
V.N. SATAKOPPAN, Department of Biochemistry, P.S.G. College of Arts and Science, COIMBATORE (T.N.) INDIA

conventional energy resource. The increase in the price of soybean and its related products internationally, makes the studies on soybean growth to be more valuable in the agricultural research. The increasing amount of cadmium in the environment affects various physiological and biochemical processes in plants (Sanitá di Toppi and Gabbrielli, 1999). Some effects of cadmium on higher plant metabolism have been investigated. Cd has been shown to inhibit enzymes of the photosynthetic calvin cycle (Van Assche and Clijsters, 1990) and enzymes involved in nitrogen metabolism (Boussama *et al*., 1999). The most pronounced effect of heavy metals in plant development is growth inhibition, which is in turn connected with cell division; however, the other mechanisms involved in those processes are still not completely understood. Tandale and Ubale (2007) have reported that the growth function such as AGR of dry matter was slow initially and it increases sharply during the period of 40th days, which is the important growth period for soybean crop. The Buttery and Buzzell (1972) found some differences in RGR varying significantly between soybean varieties. In addition, the environmental correlation indicated that plants with high NAR would be also having high RGR. Tandale and Ubale (2007) also have reported that RGR values found high during 40 to 60 days were decreased with increasing soybean crop age. A similar kind of observation by Pushpakumari *et al*. (1993) have reported an increase in NAR between 40 – 60 days and from 60 to 90 days and showed an increase in NAR under field trial for different varieties of soybean. On the other hand a contrast result have been reported by Jain *et al*.(1996) who found it decreased with increase in plant age. Tandale and Ubale (2007) also have reported a decrease in the mean-maximum NAR after the period of 30 to 45 days.

More studies are thus needed to explain the relationship of these parameters for soybean growth. Hence, in the present study we are interested in establishing a correlation between the cadmium induced changes in growth parameters absolute growth rate, relative growth rate, and net assimilation rate of Co-2 soybean and the pod yield as measured on 40th day. Hence, this study may help for a better understanding of cadmium toxicity in soybean yield, and may provide new insight in effective utilization of cadmium-contaminated water for growing soybean.

MATERIALS AND METHODS

The synthetic effluent of cadmium was prepared using cadmium chloride. A stock solution was prepared from which 5,10,15,20 and 25-ppm cadmium solutions were prepared and used for the experiment. All the solutions were prepared using double distilled water. Soybean Co-2 variety was obtained from the Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore and used for the study. Double distilled water was used to grow the control plants and wherever required throughout the study.

The seeds were initially surface sterilized using 0.1% $HgCl_2$ and washed with double distilled water for six times and used further for the studies. All the seeds were soaked in the respective cadmium solutions overnight and then transferred to plastic pots of 10 cm diameter filled with red soil. The respective amount of the cadmium solutions were calculated and added to the soil to ensure the respective cadmium concentrations in the soil and plants grown in the ordinary exposure to light (16 hrs of day and 8 hours of night), for 40 days. Five soybean plants were uprooted at 20, 40 days and at the harvest for each treatment and the following observations were recorded timely and based on collected data various growth function were calculated as follows:

The percentages of relative seed germination, and GI were calculated as referred by Tam and Tiquia (1994) and Araujo *et al*. (2001). From the data of total dry matter (g/plant) and leaf area measured per plant at 20, 40th days, the absolute growth rate was calculated by the formula given by Radford (1967) and expressed as g/day. The relative growth rate RGR is an index of the amount of growing material incorporated per unit dry weight of plant per unit time. RGR was calculated by using the formula given by Briggs *et al*. (1920) and expressed as g g^{-1} day^{-1}. Net assimilation rate is an increase in dry weight of plant per unit leaf area per unit time. NAR was calculated by using the formula given by Gregory (1926) and is expressed as g dm^{-2} day^{-1}

RESULTS AND DISCUSSION

The data required for calculation of various growth parameters on Co-2 soybean variety under normal and cadmium stress were recorded timely. The parameters such as relative seed germination percentage, germination index, total dry matter and leaf area measured per plant at 40th day and the pooled data are presented in Table 1. From these data, various growth functions AGR, RGR, and NAR were calculated and are presented in the Fig. 1, 2 and 3, respectively. The Mean pod yield on 40th day was observed and expressed as number per plant (Fig. 4). The results are discussed as under:

The AGR was calculated at 40th day for control and Cadmium treated soybean plants (cadmium concentration of 0, 5, 10, 15, 20, and 25 ppm) and are represented in Fig. 1. Observations show that the soybean mean AGR

Table 1 : Relative seed germination percentage, germination index, total dry matter and leaf area

Treatments	Relative germination percentage	Germination index	Total dry mass-40th day (gm)	Leaf area/plant -40th day (cm^2)
Control	100.00	100.00	4.36	764.64
5 ppm	89.77	55.04	2.79	465.30
10 ppm	83.89	48.05	2.42	373.92
15 ppm	71.06	32.85	1.90	267.48
20 ppm	65.20	26.74	1.69	232.92
25 ppm	57.51	21.76	1.19	198.90

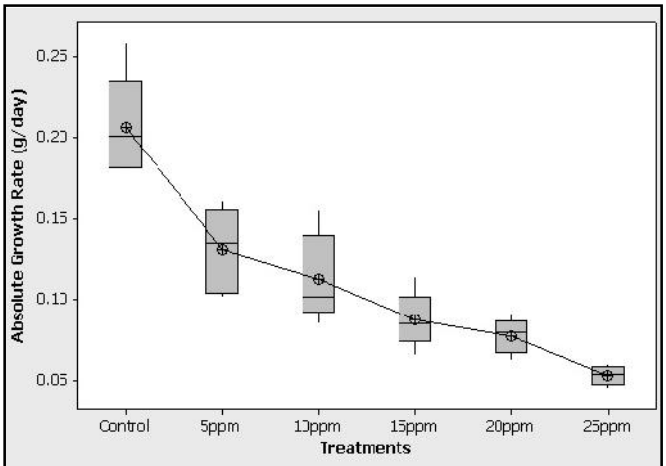

Fig. 1 : Effect of Cd treatments on absolute growth rate (g/day) on 40th day. Data represented are the means of five separate experiments, ± SE

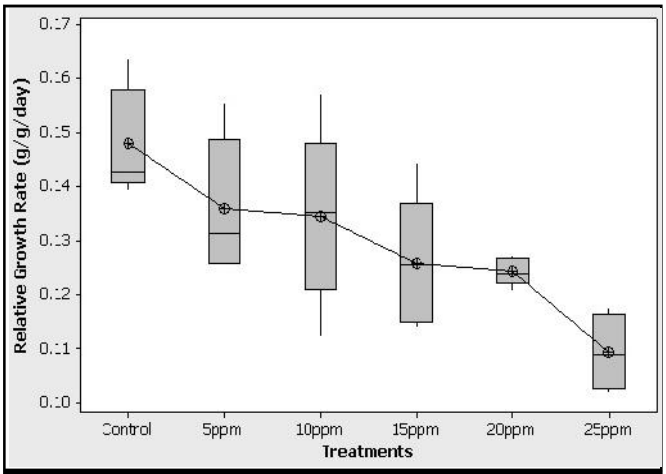

Fig. 2 : Effect of Cd treatments on relative growth rate (g/g/day) on 40th day. Data represented are the means of five separate experiments, ± SE

was found to be decreased in the plants treated with Cd than the control plants, which suggests that the seed yield may be significantly affected with the increase in the cadmium levels, as according to (Tandale and Ubale, 2007) mean LAI, AGR have a positive correlation with the seed yield. Similar results have been observed with the correlation studies of mean AGR to the pod yield on 40th day (Table 2).

The results of Mean RGR, calculated at 40th day for the control and cadmium treated plants were observed (Fig. 2). It has been reported that the mean RGR had significant positive correlation with the seed yield (Tandale and Ubale, 2007). In this study, the observations have shown a negative correlation between the increase in cadmium concentrations and RGR values, which suggested the toxic effect of Cadmium in the growth of soybean (Table 2).

The Mean NAR calculated at 40th day for the control and the cadmium treated plants were found to have varying values (Fig. 3), which might be suggesting the variation in uptake and translocation of cadmium into the soybean. It has been reported that Cd transport to the shoots represents 2% in soybean (Cataldo *et al.*, 1981). Generally, the gradient of Cd concentrations in the plant is found to be declining in the order root > leaves > seeds (Hart *et al.*, 1998), but there are some differences among

Table 2 : Studies of correlations between parameters

		Treatments	No of pods / plant	NAR	RGR	AGR
Treatments	Pearson Correlation	1	-.642(**)	.053	-.746(**)	-.877(**)
	Sig. (2-tailed)	.	.000	.780	.000	.000
No of pods / plant	Pearson Correlation	-.642(**)	1	.383(*)	.726(**)	.783(**)
	Sig. (2-tailed)	.000	.	.037	.000	.000
NAR	Pearson Correlation	.053	.383(*)	1	.485(**)	.266
	Sig. (2-tailed)	.780	.037	.	.007	.155
RGR	Pearson Correlation	-.746(**)	.726(**)	.485(**)	1	.859(**)
	Sig. (2-tailed)	.000	.000	.007	.	.000
AGR	Pearson Correlation	-.877(**)	.783(**)	.266	.859(**)	1
	Sig. (2-tailed)	.000	.000	.155	.000	.

* and ** indicates significant of values at P=0.05 and 0.01, respectively

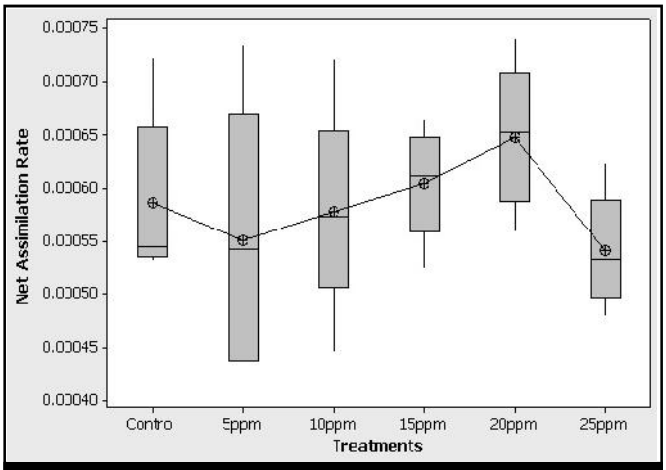

Fig. 3 : Effect of Cd treatments on net assimilation rate (g dm^{-2} day^{-1}) on 40th day. Data represented are the means of five separate experiments, ± SE

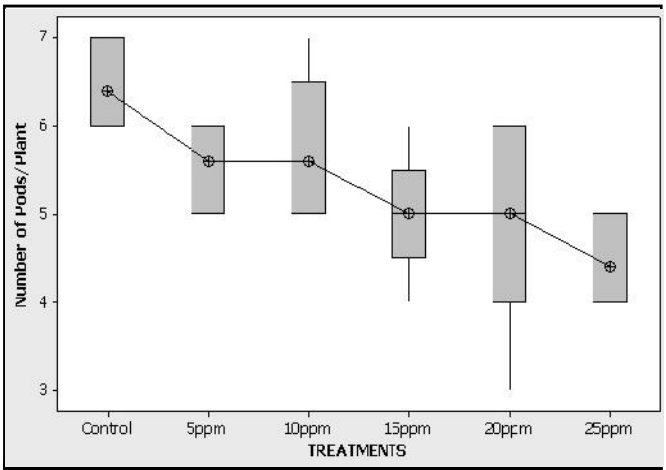

Fig. 4 : Effect of Cd treatments on mean pod yield (Number of pods/plant) on 40th day. Data represented are the means of five separate experiments, ± SE

the species and genotypes regarding Cd allocation in the shoot. It has been reported that the mean NAR has a positive correlation with seed yield (Tandale and Ubale, 2007). Present results also showed a significant correlation to the pod yield as noted on 40th day (Table 2).

Thus, it is concluded from this study that the values of AGR, RGR, and NAR at crop growth stages for soybean grown under cadmium stress were significantly altered with the increase in the cadmium stress given for the plants. The results suggested that these values could determine the seed yield of the soybeans under cadmium stress. This also shows that these values of AGR, RGR, and NAR at 40th day could be used as the indicative parameters for the reduction in the seed yields due to the increasing cadmium or heavy metal toxicity studies, which may be much helpful in deciding the use of cadmium contaminated water streams for growing these soybean plants or in deciding the respective treatment procedures. This study may help to improve the production of soybean to meet the increasing global demands and for development of effective treatment methods for reuse of wastewater for agriculture.

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