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REVIEW ARTICLE

EFFECT OF CHLOROCHOLINE CHLORIDE (CCC) ON PLANT GROWTH AND DEVELOPMENT

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ABSTRACT

The plant hormones are extremely important agent in the integration of developmental activities. Environmental factors often exert inductive effects by evoking changes in hormones in metabolism and distribution within the plant. Apart from it, they also regulate expression of intrinsic genetic potential of plants. Control of genetic expression has been demonstrated for the phytohormones at both transcriptional and translational levels. Also, hormones receptors and binding proteins have been identified on membrane surface that are specific for some hormones. Apart from nutrition, if plants hormonal balance can be made through chemical manipulation, there would be a benefit in economising the nutrients and augmenting oil seed production. One such group of chemical is thought to be plant growth regulators. Besides naturally occurring growth regulating substances, there are chemicals which may act as plant growth promoters or plant growth retardants. Chlorocholine Chloride (CCC) is gibberellin biosynthesis inhibitor involved in the inhibition of cyclization of geranyl -geranyl pyrophosphate to copyallyl pyrophosphate. But the present study indicates that chlorocholine chloride is a quaternary ammonium compound type of growth retardant involved in a diverse array of cellular, developmental and stress related processes in plants. A number of examples of the role played by CCC in the growth and development of plants are described, plant height, leaf number, leaf area index, dry mass, chlorophyll and photosynthetic parameters, photosynthetic active radiation, nutrient uptake, seed yield, biological yield, oil yield, harvest index, amino acid and protein content. The study indicates that the process of growth and development in addition to the yield of plants is significantly affected by the chlorocholine chloride in both irrigated and non- irrigated conditions.

INTRODUCTION

The chemical control of the plant growth to reduce the size through the use of plant growth regulators is a common practice to make a plant more compact and commercially more acceptable. A number of synthetic compounds are known to manage shoot growth in higher plants without being phytotoxic or causing malformation or damage (Salisbury & Ross 1994). Some of these substances have been found in agricultural practices , since they reduce the rate of stem elongation and have been shown to be involved in the regulation of photosynthesis and the movement of photosynthetic products from their site of synthesis in the leaf to the site of accumulation (Thomas, 1986; Krishnamoorthy, 1993; Khan 1996; Khan *et al.,* 2000, Lone 2001, Mir 2002 Mir *et al.* 2008; Mir *et al.,* 2009). The most commonly used and best understood group of plant growth regulators consist of those which inhibit gibberellin biosynthesis as for example quaternary ammonium compounds pyrimidines, triazoles and norbornenodiazetines that interfere with biosynthesis of gibberellins and sterols (Izum *et al.,* 1984 Rademacher and Jung, 1986; Krishnamoorthy, 1993).

Chlorocholine Chloride is gibberellin biosynthesis inhibitor involved in the inhibition of cyclization of geranyl-geranyl pyrophosphate to copyallyl pyrophosphate. Growth regulators which inhibit the biosynthesis of gibberellins have been shown to enable the plants to impart tolerance against abiotic stress due to water (De *et al.,* 1982; Knapp *et al.,* 1987; Davis and Curry, 1991). Besides this, other growth regulators of the

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same category include mepiquate chloride, AMO-1618, phosphon-D and phosphonium chloride (Hedden, 1990; Krishnamoorthy, 1993) and have extensively used in agriculture (De *et al.,* 1982; Knapp *et al.,* 1987*;* Grewal and Kolar 1990; Davis and Curry, 1991). Several triazole derivatives are known to be effective inhibitors in higher plants and thus have potential application in agriculture (Sauerbrey *et al.,* 1988; Davis and Curry, 1991; Krishnamoorthy, 1993). Triazole compounds, which include paclobutrazol, unicanozole, triapenthenol, BAS 111 and LAB 150978, inhibit the microsomal oxidation of kaurenol, and kurenal (Izumi *et al.,* 1984). It is known that the triazole type of growth regulators may affect reactions other than ent- kaurene oxidation. Tatcyclacis is a norbornenodiazetine derivative which reduces gibberellin biosynthesis by blocking microsomal oxidation of kaurene of kaurenoic acid (Rademacher and Jung, 1986) and also inhibits sterol biosynthesis, acting like triazole type plant growth retardants (Davis and Curry, 1991). Prohexadione calcium and Inabenfide also have been found to have growth retardant activity by blocking gibberllin biosynthesis (Arteca, 1997).

Crop Response to Cycocel (CCC) and Gowth parameters

1. Plant height

Growth regulators have been found to affect palnt height (Sauerbrey *et al.,* 1987; Guruprasad and Gurupasad, 1988; Djkstra and Kuiper, 1989; Kishnamooerthy, 1993). Trtiary ammonium compounds like CCC produced reduction in height without any malformation by reducing cell elongation and also by lowering cell division (Rademacher and Jung , 1986; kar *et al.,* 1989; Choudhary and Gupta *et al.,* 1996 Lone 2001).

Foliar spray of CCC reduced plant height in *Brassica napus* (Grewal *et al.,* 1993). Foliar spray of 10, 25 and 50 mg/L of uniconazole reduced seedling height in winter rape (*Brassica napus* L.), (Zhou and Ye 1996). CCC application reduced plant height in *Catharanthus roseus* L. (Choudhary and Gupta, 1996). Plants of *Brassica napus* when treated with BAS III…W via the soil at 3mg per pot , plant height was reduced to about 20 % than that of controls (Hedden *et al.,* 1989). Spraying of pepper plants with paclobutrazol reduced the plant height by 20 % as compared to control plants (Lurie *et al.,* 1994). Paclobutrazol has been used extensively to reduce seedling height in rape (Scarisbrick *et al.,*1985 ; Zhou and Xi , 1993).

Cycocel at 500ml/ ha and mepiquat chloride at 1.25 l/ha applied at flower initiation stage significantly reduced plant height of Indian mustard (Rajput *et al.,*1996).Application of CCC reduced plant height in soybean (Abo-El-Kheir, 1994), flax plants (Osman and Abu-Lila; 1985) groundnut (Jeya Kumar and Thangaraj, 1996), linseed Leitch and Kuat,1999), unflower (Pando and Srivastava , 1985), oat (Sangeeta and Varshney, 1987) buckwheat (Tahir and Farooq, 1998), barley (Sanvicente *et al.,* 1999) and *Brasicca juncea* (Lone, 2001).

2. Leaf Number

Researchers have observed suppression of leaf expansion and production of more compact plants with darker green foliage following growth retardant application (Shanahan and Nielsen, 1987; Butler *et al.,* 1989; Sairam *et al.,* 1989; Zhou and Xi, 1993; Kulkarni *et al.,* 1995; Reddy *et al.,* 1996; Zohu and Ye, 1996 and Lone 2001).Total number of leaves per plant of *Catharanthus roseus* L. increased with the application of CCC over control (Choudhary and Gupta, 1996). Singh (1996) found that application of CCC increased panicle number per plant in wheat genotypes. However, in soybean plants treatment with CCC significantly decreased number of leaves per plant (Abo- El- Kheir *et al.,* 1994). It is also reported that CCC application decreased the number of leaves per plant in *Arachis hypogea.* Foliar sprays of unicanozole at 10, 25 and 50 mg/litre concentration significant by increased the number of green leaves in winter rape (Zhou and Ye, 1996).

3. Leaf area

Leaf area per plant was found to increase significantly by cycocel treatment at 1500 mg/l over control in *Cyamposis tetraganoloba* L. Singh (1996) found that exogeneous application of CCC increased leaf area per plant in gaur cultivars (Afria *et al.,* 1988). Contrary to this some reports say that exogenous application of CCC significantly reduced the total leaf area in plants like soybean cultivars (Abo – El – Kheir *et al.,* 1994) cotton (Reddy *et al.,* 1996) sunflower (Pando and Srivastava, 1985) and *Brassica juncea* (Lone 2001).

4. Leaf area index

Jeya kumar and Thangaraj (1996) found that CCC reduced leaf area index in groundnut. CCC application also reduced LAI in *Brassica napus* (Grewal *et al.,* 1993). However Grewal and Kolar (1990) could not find a change in response to the application of CCC (250ppm) on *Brassica juncea*.

5. Dry mass

Exogenous application of CCC increased dry matter production in rape (*Brassica juncea*). Increase in dry matter percentage by CCC application was also reported by Dijkstra and Kuiper (1989). Choudhury and Gupta (1996) reported increased dry weight of leaves per plant of *Catharanthus roseus L.* with application of CCC. Plants of *F sagittatum* receiving 100mg/L Cycocel showed an increase in dry matter (Tahir and Farooq, 1998). Exogenous application of CCC increased dry weight of shoot in green gram (Mandal *et al.,* 1997).Contrarily Abo-El- kheir *et al*. (1994) reported decrease in dry weight of soybean plants on application of CCC. Ghosh *et al*. (1994) observed increased dry weight per plant in sesame, safflower and linseed by the application of growth regulators like Mirculan, Nutron, Planofix, and Paras over control plants. Mepiquat chloride application also enhanced total dry matter production in groundnut (Jeyakumar and Thangraj, 1996). Application of 6B1 decreased dry weight of corn (Kasele *et al.,* 1995), but unicanozole treatment increased dry weight of rape (Zhou and Ye, 1996). Exogenously applied CCC at 400ppm concentration significantly increased the dry mass of *Brassica juncea* cultivars (Lone 2001).

6. Crop growth rate (CGR), relative growth rate (RGR) and net assimilation rate (NAR)

Exogenous application of CCC and mepiquat chloride enhanced the crop growth rate of groundunt (Jeyakumar and Thangraj, 1996). However, RGR was decreased by CCC application in *Plantgo major* (Dijksrtra and Kiuper, 1989). While 400ppm of CCC exogenously applied enhanced both CGR and RGR of *Brassica juncea* under non -irrigated conditions (Lone 2001).

Cyocel and photosynthesis parameters

1. Chlorophyll

Favourable effects of growth regulators on chlorophyll content have been reported by number of workers. Cyocel at 250 and 500 ppm significantly improved the chlorophyll contents in leaves of *Brassica napus* (Grewal *et al.,* 1993). The beneficial effects of cycocel n chlorophyll content have also been reported in various systems, like mungbean (Shah and Prathapasenan, 1991) green gram (Mandal *et al.,* 1997) varieties of guar (Afria *et al.,* 1998), sesamum (Bashist, 1990), soyabean (Abo-El-Kheir *et al.,* 1994), onion (Miroshnichenko and Manankov, 1992), wheat (Sairam *et al.,* 1991), safflower (Kar *et al.,* 1989), *Brassica juncea* (Lone 2001) and cotton (Kumar *et al.,* 2005).

2. Photosynthesis

Increased rates of photosynthesis per unit leaf area have been observed after the application of growth regulators on different plant species (Liu *et al.,* 1993; Yang *et al., 1994*). Plant growth regulators can affect photosynthetic CO_2 uptake either by affecting stomatal aperture or by affecting the activity of photosynthetic enzymes (Foroutan –pour *et al.,* 1997). Exogenously applied CCC as spray increased the photosynthetic rate in pigeon pea (Dayal *et al.,* 1993). Cycocel has been found to cause stimulation in the rate ribulose biphospahte carboxylase activity and rate of CO_2 fixation (Numi, 1979; Pando and Srivastava, 1985). CCC application recorded higher hill reaction activity and total chlorophyll contents indicating higher photochemical in genotype of rice (Bashist, 1990) under moisture stress as well as irrigated conditions. CCC application to foliage of wheat increased the photosynthetic rate (Sairam *et al.,* 1991). The beneficial effect of CCC on photosynthesis, chlorophyll and nitrogen metabolism under moisture stress have also been reported by others (Avundhzyan and Shirakyan, 1974; Kisamutdinova *et al.,* 1974; Dayal *et al.,* 1993; Lone 2001 and Kumar *et al.,* 2005). Butler *et al.* (1989) found that the use of bioregulators had little effect on the photosynthetic rate of leaves which were fully expanded at the time of application , but for leaves which developed subsequently, there was a reduction in photosynthesis per unit chlorophyll. The reduction in the rate of photosynthesis was explained as because of decreased stomatal conductance. Application of both ABA and triadimefon considerably enhanced rate of photosynthesis in wheat under moisture stress condition (Sairam *et al.,* 1989). Enhancing effect of other growth retardants mixtalol (Zhou and Xi, 1993) and uniconazole (Zhou and Ye, 1996) were also recorded on photosynthesis.

3. Photosynthetically active radiation

Photosynthetically active radiation (PAR) is a measure of radiation available for photosynthesis. It is well known that plants vary in response to radiations of different wave lengths with in the canopy. Mean sunlight irradiance or the proportion of sunlight leaf surface diminishes as exponential function of leaf area index. Changes in radiation quantity also occur largely due to the spectral properties of leaf pigments, leading to a reduction in the red/far red ratio as light penetrates the canopy. In this respect there are several evidences that potentiate the claims of canopy change under the influence of growth regulators, which bring about a desirable modification in PAR. Grewal and Kolar (1990) in their experiment on *Brassica napus* reported that application of CCC (250 and 500ppm) had negative impact on PAR interception. Lone (2001) also reported PAR decreased with increasing levels of cycocel in *Brassica juncea* cultivars under non - irrigated conditions.

Cycocel and nutrient uptake

Plant growth regulators are known to influence ion transport, have special effects on membrane properties and on transport functions. Growths regulators have affiliated with reinforcement of assimilate translocation in established sink -source systems (Thomas, 1986). Desirable increase in the produce of field crops was due to alteration in the trends of assimilate distribution (Addo-Quaye *et al.,* 1986). The allocation of newly fixed carbon into different metabolic products influenced the partitioning of carbon and growth activity of the whole plant. It is reported that K level in the stems of soybean (*Glycine max*) increased on application of CCC grown under green house conditions. Foliar application of chlorocholine chloride at the rate of 300ppm at the flower initiation stage increased the uptake of N, P and K in soybean plants. N uptake by CCC treatment was reported in sesamum (Bashist, 1990). Chlormequat application increased uptake of N, P and K in *Brassica juncea* (Guroo and Patel, 1993). Exogenous application of CCC enhanced nitrogen content in tall genotype of wheat (Singh, 1996) and sesamum (Bashist, 1990). It was explained that CCC affected the membrane leading to reduced K^+ in guard cells and causing stomatal closure .Exogenous foliar application of 400 ppm cycocel enhanced the uptake of nitrogen , phosphorus and potassium in *Brassica juncea* cultivars under non- irrigated conditions (Lone 2001).

Cycocel and yield parameters

Yields components like pod number, seed number per pod and seed weight do not only depend on nutritional factor but also on hormonal status (Morgan, 1998; Crosby *et al.,* 1981; Carlson *et al.,* 1987; De-Bouille *et al.,* 1989; Paulpandi *et al.,* 1998).

1. Pod Number

Foliar application of chlorocholine chloride at the rate of 300ppm at the flower initiation stage improved the number of pods per plant in soybean (Sing *et al* 1987). Chlormequat application increased the number of pods per plant in *B. juncea* (Ashraf *et al.,* 1987; Saini *et al.,* 1987; Lone, 2001), ground nut (Jeya Kumar and Thangraj 1996), soyabean (Abo-El Kheir *et al.,* 1994), green gram (Mandal *et al.,* 1997).

2. Seed Number

Pod number and seed number per pod are determined early after flowering (Pechan and Morgan, 1983) and have been found to be influenced by growth regulators (Zhou and Xi, 1993; Foroutan –pour *et al.,* 1997). Cycocel application influenced the number of seed/capsule positively in linseed plants. Abo El Kheir *et al.* (1994) reported increased seed number per plant in CCC treated soybean cultivars. Further, chlormequat application increased the number of grains per year by 26-29 % in wheat and number of bolls located per plant in cotton crop (Cia *et al.,* 1996). However, Ashraf *et al.* (1987) found the effect of CCC on number of seeds per pod in *Brassica juncea* to be non- significant .Similar results were also reported by Lone (2001) on *Brassica juncea* cultivars under non- irrigated conditions.

3. 1000 seed weight

Seed weight of the crop is noted to be influenced with the application of plant growth regulators. Saxena *et al.* (1991) found that CCC (200ppm) contributed towards a large increase in 1000 seed weight of Sesamum indicum L. Saini *et al.,* (1987) and Tripathi and Singh (1989) in *Brassica juncea* L., Cia *et al. (1996)* in cotton , Kumar and Bharti (1988) and Pando and Srivasatava (1987) in sunflower reported an increase in seed weight due to CCC. In contradiction to above results Ashraf *et al.* (1987) and Lone (2001) found no significant effect of CCC on seed weight of *Brassica juncea.* Reduction in seed weight was noted by Lucas *et al*. (1993) in spring wheat cultivars and in *Brassica napus* by Grewal *et al.* (1993).

4. Seed yield

Improvement in the seed of *Brassica juncea* in response to the chlormequat was observed by Ashraf *et al.,* (1987) , Saini *et al* (1987), Singh *et al*. (1988), Tripathi and Singh (1989), Guroo and patel (1993), and Rajput *et al.* (1996). Application of CCC has been found to influence the total seed yield / plant positively in linseed (*Linum usitatissimum*), *Cyamospsis tetragonoloba* L. (Afria *et al.,* 1998), green gram (Mandal *et al.,* 1997), triticale (Naylor and Stephen, 1993), corn (Shanahan and Nielsen, 1987), wheat (Knapp *et al*. 1987; Sairam *et al.,* 1991; Lucas *et al.,* 1993), sunflower (Pando and Srivastava, 1985; Kumar and Bharti, 1988 sesame (Tripathi and Singh, 1989; Saxena *et al.,* 1991), soybean (Abo-El-Kheir *et al.,* 1994) ground nut (Gridhar and Giri, 1997). But contrary to these results, application of paclobutrazole to oilseed rape could not induce any increase in the seed yield of the crop (Scarisbrick *et al.,* 1985). Grewal *et al.* (1993) observed that CCC of 500ppm concentration substantially decreased seed yield in *Brassiac napus*. While Lone (2001) observed that 400ppm concentration of exogenously applied CCC increased seed yield substantionally in *Brassica juncea* under non-irrigated conditions.

5. Biological yield

Exogenous application of CCC increased the biological yield in different genotypes of wheat (Singh, 1996) and *Brassica juncea* (Lone 2001).

6. Harvest index

An application of cycocel has shown increase in harvest index in sunflower (Pando and Srivastava, 1985), wheat (Singh, 1996) *and Brassica juncea* (Lone 2001). However, Afria *et al*. (1998) in varieties of *Cyamopsis tetraganoloba* L. reported significant decline in harvest index due to cycocel treatment.

7. **Oil yield**

Remarkable enhancement in the oil yield winter rape (*Brassica napus* L.) was recorded when uniconazole (Zhou and Ye, 1996) mixtalol and paclobutrazol (Zhou and Xi, 1993) were applied. Cycocel spray of 400 ppm was found to have highest value for oil yield in different cultivars of *Brassica juncea* under non- irrigated conditions (Lone 2001).

Cycocel and quality parameters

1. Oil content

The oil content in the seeds of sunflower (*Helianthus annus* L.) increased by the application of CCC applied either at pre or post flowering satge (Pando and Srivastava, 1987). Seed oil content of *Brassica juncea* also increased by chlormequat application (Tripathi and Singh, 1989). Saini *et al*. (1987) also reported similar results in *Brassica juncea* under both irrigated and rainfed conditions. However, Grewal and Kolar (1990), Guru and Patel (1993) and Ashraf *et al*. (1987) in *Brassica juncea*, Grewal *et al*. (1993) in *B. napus* L., Leitch and Kuat (1999) in linseed, Abo-El-kheir *et al*. (1994) in soybean and Lone (2001) in *Brassica juncea* reported that exogenous aplicatoin of CCC had no significant effect on oil content of the seeds. Contrarily, Osman and Abu - Lila (1985) in linseed reported that application of CCC decreased seed oil content.

2. Amino acid content

In a field trail with triticale, Nayler and Stephen (1993) recorded that early application of CCC increased the proportions of aspartic acid, histidine and alanine, while leucine content was decreased. However, late application had no effect on the proportions of histidine, but increased aspartic acid, glutamic acid leucine. Jain and Guruprasad (1989) observed an increase in the level of free amino acids in particular phenylaladenine in seedlings of radish treated with CCC . Choudhary and Gupta (1996) observed that application of CCC brought about an increase in the free amino acid content in *Catharanthus roseus* L.

3. Protein Content

Plant growth regulators have been found to influence significantly the protein content in crop plants (Liu *et al.,* 1993; Lurie *et al.,* 1994; Yang *et al.,* 1994; Kulkarni *et al.,* 1995). Growth regulators CCC application increased protein and RNA content of leaves in safflower (*Carthamus tinctorius* L.) (Kar *et al.,* 1989), protein content in soybean seeds (Abo– El- Kheir *et al.,* 1994) and protein content of B. *juncea and B. napus* .Treatment with CCC (250ppm) to oat (Avena stiva L.) resulted in enhancement in the crude protein content.

4. Fatty acid

Plant growth regulators treatments which included chloromequate at any early growth stage of linseed altered the relative proportions of fatty acids, reducing the content of linolenic acid while increasing that of oleic acid (Leitch and Kuat, 1999). In linseed also the levels of oleic acid were increased while those of linolenic acid were generally decreased by cycocel application (Osman and Abu-Lilla, 1985). In ground nut total lipids and triglycerides were increased by the application of cycocel. CCC treatment has also been found to increase fat content of soybean seeds. Carbaryl application increased triglyceride content as its concentration increased but the rate of beta oxidation of fatty acids and the activities of glyoxylate cycle enzymes isocitrate layse and malate synthetase decreased in *Brassica niger* (Chakrabarti *et al.,* 1990).

CONCLUSIONS

This study highlights the pivotal part played by the hormone in the form of chlorocholine Chloride (CCC) on plant growth and development by affecting various growth parameters like: plant height, leaf number, leaf area, leaf area index, dry mass, crop growth rate, relative growth rate, net assimilation rate; photosynthetic parameters like chlorophyll, photosynthesis, photosynthetically active radiation, nutrient uptake in addition to yield parameters like; pod number, seed number, 1000 seed weight, seed yield, biological yield, harvest index, oil yield and quality parameters like oil content, amino acid content, protein content and fatty acid content.

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