Growth parameter study in *Camelina sativa*, a potential biofuel crop, under *in-vitro* culture conditions

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ABSTRACT

The present study was conducted with the aim to find the effect of *in vitro* culture conditions on growth parameters, viz. germination, growth vigour, cotyledon opening, true leaf emergence, plant water content, shoot fresh weight, dry weight and leaf weight ratio of *Camelina sativa* (cv Calena). It was found that there was no significant difference with respect to germination (almost 100%) in all the media (water agar, half MS and full MS) but the rate of germination measured as Timson index significantly differed. In water agar the rate of germination was 95.42% in full MS 86.9% and in half MS 83.33%. Emergence of true leaf and growth vigour was highest in full MS as compared to half MS and water agar. There was significant difference in rate of germination, cotyledon opening, true leaf emergence, plant water content, fresh weight, dry weight and leaf weight ratio in all media. Shoot length was maximum (4.5 cm) in MS medium with highest fresh weight and dry weight (0.35 mg; 0.023 mg) as compared to half MS (0.204 mg; 0.017 mg) and water agar (0.031 mg; 0.014 mg). When explants (hypocotyl, leaf segment, nodes and shoot tip) collected from *in vitro* seedlings were cultured in MS with 1-5 mg/l BAP, 4 BAP gave best response for shoot tip and nodal explants followed by other combinations in descending order (100%, 88%, 82%, 77%, 73% in 4 BAP, 5 BAP, 3 BAP, 2 BAP and 1 BAP respectively). Other explants responded by enlargement and curling with little or no callus and eventually turned brown and necrotic. The preliminary results obtained in the present study shows the *in vitro* response of *Camelina sativa*, which can be used further for *in vitro* abiotic stress studies and genetic manipulation studies.

Key words: Camelina, Germination, Media, Regeneration, Sterilization, Tissue culture

*Camelina sativa* L. Crantz (Zubr 1997, Ryhanen et al. 2007, Buchenschutz-Nothdurft et al. 1998, Lu and Kang 2007). Recently generated interest to scientists and oil processors as a crop for diversification of agriculture, and as a source for production of high quantity and quality oil for biofuel, feed, food, and pharmacy (Grauda et al. 2007). For energy security, *Camelina sativa* species (other species are: *Camelina alyssum*, *Camelina microcarpa*, *Camelina rumelica*) belonging to the flowering plant family Brassicaceae is being explored for its potential as biofuel plant (Al-Shaehbaz 1987). It is native to Mediterranean regions of the world like, Europe, Asia, Turkmenistan, Afghanistan, Pakistan, Iran and Spain (Crowley and Frolich 1998). It is also known by other names such as German sesame, false flax, gold-of-pleasure, and Siberian oilseed. It is known to harbour cold-hardiness, drought tolerance and can grow on marginal crops. Camelina has very low requirements for tillage and weed control. Camelina needs little water or nitrogen to flourish; it can be grown on marginal agricultural lands and does not compete with food crops. *Camelina sativa* is an annual short season crop, reaching maturity in 85-100 days. Plant grows to a height of 1-3 feet with branching stems, which become woody at maturity (Ehrensing and Guy 2008). It is gaining importance for being able to withstand water shortages in early stages of development and is a low input crop (Pilgeram et al. 2007, Lu 2008, Perkins 2010). Camelina oil has unique properties. Camelina oil is rich in omega-3 fatty acid, alphalinolenic acid (45% ALA) (Zubr 2002). The oil contains about 64% polyunsaturated, 30% monounsaturated, and 6% saturated fatty acids (Schuster and Friedt 1995). The oil also contains high levels of gamma-tocopherol (vitamin E), which confers a reasonable shelf-life without the need for special storage conditions (Abramovic and Abram 2005). Camelina oil seed meal has significant crude protein content (Leonard 1998). Medical research indicates that a diet abundant in omega-3 fatty acids is beneficial to human health. Camelina is highly resistant to black leg and *Alternaria brassicace*. It is resistant to flea beetles. Research on this crop is still in its infant stage. *Camelina sativa* also has breeding importance since, it is a storehouse of useful genes, which has not been much explored. There is scanty information on tissue culture responses in Camelina. Studies in this direction become important when some genetic manipulation has to be carried out for oil enhancement or other economic traits. It also becomes important when some abiotic stress studies (salt, drought, metal) have to be carried out. The current study was carried out with the objective to

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find the effect of in vitro culture conditions on growth parameters, viz. germination, radicle protrudance, growth vigour, cotyledony leaf opening, true leaf emergence, plant water content, shoot fresh weight, dry weight and leaf weight ratio of Camelina sativa (cv Calena).

MATERIALS AND METHODS

Camelina sativa strain Calena (EC) was used in the present study. Nucleus seed of this strain was provided by Dr. Vollmann, Austria and introduced through NBPGR, New Delhi, multiplied at DIBER field station, Pithoragarh.

Camelina seeds are very minute and covered with mucilaginous coating, which imbibes water and swell upon soaking in water. Seeds were washed with water with Tween 20 and sterilized under asceptic conditions in laminar air flow hood. To sterilize optimum time for effective sterilization seeds were treated for 1, 3 and 5 min with 0.1% HgCl₂ (mercuric chloride) followed by 30 treatment with 70% ethanol followed by 3-4 washes of sterile distill water. Seeds were blot dried and cultured in water agar (WA), half MS (HMS) and full MS (FMS) media (Murashige and Skoog 1962) for germination. The pH of the media was adjusted to 5.8 before adding agar and was autoclaved at 15 psi at 121°C for 20 min.

All the cultures were maintained at 24 ± 2°C in 16 hr light and 8 hr dark cycle (cool white florescent light, 30 μmol/m²s) and 50-60% relative humidity (RH) culture room. In vitro seedlings (hypocotyl, leaf segment, nodes and shoot tip) were inoculated in MS supplemented with 1-5 mg/l BAP in sterile petriplates. Sub-culturing was done after 30 days.

Data on in vitro seed germination was recorded in terms of radicle emergence and opening of cotyledony leaves every day till germination was complete. The rate of germination was estimated in terms of Timson’s index of germination velocity as, Timson’s index = \(\Sigma (G/t)\); where G is germination percentage; \(t = \) total period of germination (days). The plants growing under different media (MS, half MS and water agar) were uprooted carefully, washed with distilled water and then the fresh weight and dry weight of leaves and shoots were recorded to find water content. The samples were oven dried at 94°C for 24 hr and dry weight was recorded.

Germination percentage and vigour was recorded for each treatment with 10 replications per treatment of 20 seeds each and 3 replicates per treatment for water content, fresh and dry weight. The program CropStat for Windows (7.2.2007.2 module), developed by the Biometrics unit, IRRI, Philippines was used for analysis of variance (ANOVA) of experiments laid out in Completely Randomized Design (CRD). The treatment means were compared by using Least Significant Difference test (LSD) at a significance level of \(P < 0.05\)

RESULTS AND DISCUSSION

Camelina seed sterilization was most effective when treated with 0.1% HgCl₂ for 3 min compared to other treatments (Table 1). No contamination was observed in 3 min and 5 min treatment. In 1 min treatment there was 30% contamination. The 100% germination was observed in water agar medium but there was difference in germination rate which was measured by Timson index. Seeds of C. sativa cultured on MS media, half MS media and water agar medium showed varied response (Table 2). All the treatments (Full MS, Half MS and WA) resulted in 100% seed germination. However, the rate of germination significantly differed. Growth vigour was highest in MS medium (Table 2) (3 min sterilization) (Fig 1) followed by half MS. In WA germination rate was 95.4% as compared to full MS 86.9% and half MS media 83.3%. On the other hand opening of cotyledon and true leaf emergence was 87.83% and 0.89% in water agar, 74.88% and 74.25% in MS and 73.22% and 70.90% in half MS media (Table 3). Plant height was highest in full MS (4.53 cm) followed by half MS (3.07 cm) and was least in water agar (0.6 cm) (Fig 2). In water agar after 6 days of germination there was no further growth response of plants as a result the height was stunted. Seedling vigour, fresh and dry weight biomass reduced in water agar due to lack of nutrients. Growth vigour was best in MS medium because it is highly enriched with macro and micro-elements with different vitamins compared to water agar medium. Though germination took place in water agar medium but there was no true leaf emergence due to the unavailability of nutrient. Because of no true leaf emergence in water agar the seedling turned...
brown and died after some days (Table 3). Plant water content was 93.4% in full MS, 91.5% in half MS and 52.9% in water agar. Plant fresh weight (mg) was 0.354, 0.204, 0.0307 and dry weight was 0.023, 0.0173 and 0.0143, (Table 4) in full MS, half MS and in water agar, respectively.

Effect of different media was evident as seed germination, seedling growth, fresh weight and dry weight was significantly different.

When explants (hypocotyl, leaf segment, nodes and shoot tip) collected from in vitro seedlings were cultured in MS medium supplemented with 1-5 mg/l BAP, 4 BAP gave best response for shoot tip and nodal explants followed by other combinations in descending order (100%, 88%, 82%, 77%, 73% in 4 BAP, 5 BAP, 3 BAP, 2 BAP and 1 BAP respectively). Shoot tip and nodal explants gave rise to multiple shoots (average 12) giving a bushy appearance. There was induction of floral buds in these shoots. Other explants responded by enlargement and curling with little or no callus and eventually turned brown and necrotic. Under in vitro conditions the plant takes short time to flower (1 month) compared to 2 months under field condition. The findings can be useful for genetic manipulation studies in vitro and also for doubled haploid generation after hybridization. This might also be useful for carrying out ovary rescue after wide hybridization.

Camelina is an under-exploited crop species with high multiplication ratio, drought and frost tolerance and short maturity period. Recent interest in this under-exploited crop mainly due to the demand for alternative low-input oil crop has led to the crop improvement studies (Vollmann et al. 1996 and 2005, Johnson 2007). Research reports across the globe have exhibited useful genetic variation which offers opportunity for further improvement in various traits (Agarwal et al. 2010). Camelina being a cold season crop, its cultivation is not possible in other seasons. It takes less time, space and also is independent of season. The current study was carried out in the month of August-September which is not the natural growing season of camelina in India. The preliminary results obtained in the present study shows the in vitro response of C. sativa, which can be used further for abiotic stress studies irrespective of the season in which it is grown and for genetic manipulation studies to further improve the seed oil content using modern biotechnological tools, making it a more attractive crop for industrial purpose.

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REFERENCES