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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, Buchsenschutz-Nothdurft *et al*. 1998, Lu and Kang 2008) 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 Frohlich 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, alpha-linolenic 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 brassicae*. 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, cotyledonary 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% $HgCl_2$ (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 (Murasighe 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/m^2s) 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 cotyledonary 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 =Σ (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% $HgCl_2$ for 3 min compared to other

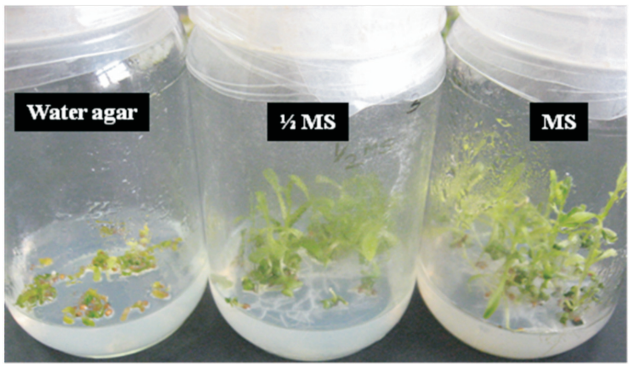

Fig 1 *In vitro* germination of C. sativa in water agar, half MS and full MS media

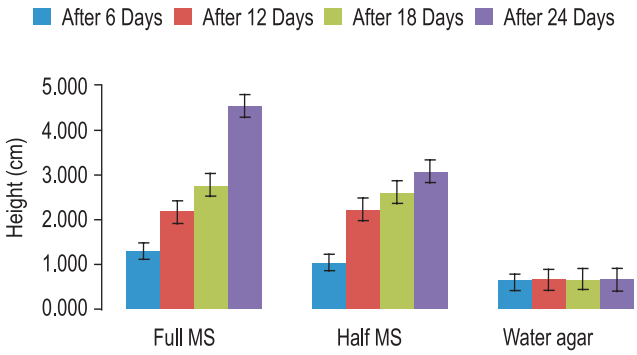

Fig 2 *In vitro* effect of MS, half MS and water agar on seedling height at different interval

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 all the different germination media 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

Table 1 Effect of sterilization treatment duration on contamination reduction and sterilization treatment duration as well as media on germination in *C. sativa* seeds.

Media/treatment	1 min	3 min	5 min
Water agar	Poor growth vigour. There was radicle emergence and cotyledon opening but no true leaves emerged.		
Half MS	Good***	Best vigour****	Good**
Full MS	Good****	Best vigour*****	Very good***

Table 2 Effect of sterilization treatment and media on growth vigour (* indicates vigour) in *C. sativa* seeds.

Media/treatment time	Contamination reduction (%)			Germination (%)		
	1 min	3 min	5 min	1 min	3 min	5 min
Water agar	30			70	90	90
Half MS				98	98	96
Full MS				100	100	98

Table 3 Effect of treatment on rate of germinaton percentage, cotyledonary opening and true leaf emergence in *C. sativa*.

Treatment	Germination (%)	Cotyledon opening (%)	True leaf emergence (%)
Full MS	86.96 b	74.88b	74.25c
Half MS	83.33 a	73.22a	70.90b
Water agar	95.42 c	87.83c	0.890a
CV	9.437	9.72	10.80
SE	1.256	1.21	1.066
LSD (P d”0.05)	0.864	0.839	0.733

*Values within experiments indicated by same letter are not significantly different at P=0.05

Table 4 Effect of treatment on (mean) leaf fresh weight, dry weight and water content (%) and leaf weight ratio in *C. sativa*

Treatment	Fresh biomass (mg)	Dry biomass (mg)	Water content (%)	Leaf weight ratio
Full MS	0.354c	0.023c	93.40b	7.60c
Half MS	0.204b	0.017b	91.50b	6.40b
Water Agar	0.031a	0.014a	52.90a	2.90a
SE	0.015	0.001	3.176	0.83
LSD (P ≤ 0.05)	0.011	0.001	2.354	0.615

*Values within experiments indicated by same letter are not significantly different at P=0.05

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. *In vitro* experiment offers several advantages over field experiments. 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

Abramovic H and Abram V. 2005. Properties of *Camelina sativa* oil. *Food Technology and Biotechnology* **43**(1)**:** 63–70.

Agarwal A, Pant T and Ahmed Z. 2010. *Camelina sativa*: A new crop with bio-fuel potential introduced in India. *Current Science* **99**(9): 1 194–5.

Al-Shaehbaz I A. 1987. The genera of Alysseae (Cruciferae; Brassicaceae) in the Southeastern United States. *Journal of the Arnold Arboretum* **68:** 185–240.

Büchsenschütz-nothdurft A, Schuster A and Friedt W. 1998. Breeding for modified fatty acid composition via experimental mutagenesis in *Camelina sativa* (L.) Crtz. *Industrial Crops*

and Products **7:** 291–5.

Crowley J G and Frohlich A. 1998. Factors affecting the composition and use of C*amelina*. Teagasc *Project* Report 4319, Crops Research Centre. Teagasc, Dublin, Ireland.

Ehrensing D and Guy S. 2008. *Camelina*. http://extension.oregonstate.edu/catalog/pdf/em/em8953-e.pdf [accessed on 27 Jun. 2009].

Grauda D, Lapina L, Stramkale V and Rashal I. 2007. *Camelina sativa* as a crop for diversification of agriculture and as a producer of high qualityoil. *Environment Technology, Resources, Proceedings* 262–5.

Johnson D. 2007. *Camelina*: An emerging crop for bioenergy, *In vitro* biology meeting abstract issue, (*In*) *Vitro Cellular and Developmental Biology* **43:** 12–23.

Leonard E C. 1998. Camelina oil: a-linolenic source. *Inform* **9:** 830–8.

Lu Chaofu. 2008. Camelina sativa: A potential oilseed crop for biofuels and genetically engineered products. *Information Systems for Biotechnology* 7–9.

Murashige T and Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiology Plantarum* **15**(3): 473–97.

Perkins J. 2010. Defence biofuels- U.S. Navy tests biofuel blend in the 'Green Hornet' jet. *Biofuel Journal* second quarter: 74.

Pilgeram A L, Sands D C, Boss D, Dale N, Wichman D, Lamb P, Lu Chaofu, Rick B, Kirkpatrick M, Thompson B and Johnson D L. 2007. *Camelina sativa*, A Montana omega-3 and fuel crop. 129–31.

Ryhanen E L, Perttil A S, Tupasela T, Valaja J N, Eriksson C and Larkk A K. 2007. Effect of *Camelina sativa* expeller cake on performance and meat quality of broilers. *Journal of the Science of Food and Agriculture* **87**: 1 489–94.

Schuster A and Friedt W. 1995. *Camelina sativa*: old face – new prospects. *Eucarpia Cruciferae Newsletter* **17**: 6–7.

Vollmann J, Damboeck A, Eckl Schrems H and Ruckenbauer P.1996. Improvement of *Camelina sativa,* an under-exploited oilseed, pp 357–362. (*I*n) J. Janick (ed.) Progress in New Crops, ASHS Press, Alexandria, V A.

Vollmann J, Grausgruber H, Stift G, Dryzhyruk V and Lelley T. 2005. Genetic diversity in camelina germplasm as revealed by seed quality characterstics and RAPD polymorphism. *Plant Breeding* **124**: 446–53.

Zubr J and Matthaus B. 2002. Effects of growth conditions on fatty acids and tocopherols in *Camelina sativa* oil. *Industrial Crops and Products* **15**: 155–62.

Zubr J. 1997. Oil-seed crop: *Camelina sativa*. *Industrial Crops and Products* **6**: 113–9.