

Effect of Root Pruning and Nitrogen Application on Growth Performance of *Celtis australis* L. Seedlings in Garhwal Himalaya, Uttarakhand

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Abstract: The present study was conducted at College of Forestry, Ranichauri – Tehri Garhwal (UK) to assess the effect of root pruning and nitrogen application on the growth performance of *Celtis australis* seedlings. The seedlings roots were pruned at 0, 6 and 12 cm from the collar region and nitrogen was applied @ 0, 50, 100 and 150 Kg ha⁻¹. The growth performance, physiological and nutrient attributes *viz.* shoot length, root length, collar diameter, number of leaves per plant, dry weight of shoot, root and leaves, survival percent, white root regeneration, chlorophyll content and NPK content and uptake of *Celtis australis* seedlings were significantly enhanced with root pruning and nitrogen application. The root pruned at 12 cm from collar region and application of nitrogen @ 100 Kg ha⁻¹ gave the best results for growth of *C. australis* seedlings. Whereas, the seedlings of control treatments of both the factors showed the least values for growth and physiological attributes.

Keywords: Root pruning, Nitrogen application, Growth performance, Physiological, Nutrients attributes

Celtis australis L. is an important multipurpose tree species of Garhwal Himalaya.It is mainly grown for fodder, fuel, timber and plays a very important role in socioeconomic structure in hilly areas. Its leaves are highly palatable, nutritious and tannin-free and are available during the period of scarcity of green fodder (Yadav and Bisht 2013). The wood of *C. australis* is also suitable for carving and making musical instruments, sports equipments and paddles (Brus 2005). It can be grown in any type of soil, well-drained loamy, sandy soil and also on dry gravels soil and is found very drought resistance once established (Komarov 1968). However, poor germination of seeds in nursery was recorded and one year old seedlings have been found suitable for the plantation in field condition (Singh et al 2006).

Roots are one of the most important plant organs which are commonly found under the soil with the positive gravitropism. Compared to the above ground, plant parts such as stems or leaves which get more attention, roots get less attention because their existence is underground or below soil. Roots play an important role in supporting the plant for better growth or for long time survival of plants, providing support to stems and shoots above the ground and also supporting nutrients and water absorption under the ground and serving as nutrient storage in some species (Tjitrosoepomo 2009). A good and well established portion of the root system to shoot system in plants is more important, which can be attained by the root pruning techniques. Root pruning is one of the most familiar and important practice of removing the small portion of roots from the plants root system (Chauhan et al 1994a,b, Gazal et al 2004). Root pruning is a technique to regenerate and nurture tree size including canopies and roots for attaining to maximum production (Marini 2014).

Plants need nutrients to grow and fertilizers are a good source of nutrients. Nitrogen is the most important nutrient element which is essential for the endurance of all livelihoods (Rosolem 2017). Nitrogen (N) has an impact on all aspects of plant function in growth, development and from metabolism to resource allocation (Yousaf et al 2021). Generally fertilizers are used to maintain and influence the nutritional circumstances of different cropping structure. Once nitrogen fertilizers are applied to cropping systems, the fertilizers are directly absorbed by plants root system (Liu et al 2014). Fertilization is also reported to increase leaf nutrient, dry matter, fruit yield and chlorophyll content in plants (Raese et al 2007).

In the hills, transportation of root pruned seedlings in comparison to transportation of containerised grown seedlings from nursery to plantation sites reduces transportation costs and better establishment. The nitrogen application may further be beneficial for the quick establishment of seedlings. Thus, keeping in view above points, present study is carried out to study the performance of one year old *C. australis* seedlings in response to root pruning and nitrogen application.

MATERIAL AND METHODS

Experimental site The experiment was conducted at Forest Nursery, College of Forestry, Ranichauri - Tehri Garhwal, Uttarakhand, India located at an altitude of 1850 masl and lies between 30°15" N latitude and 78° 30" E longitude. The average monthly minimum temperature ranged from 0.6°C to 15.4°C while, average maximum temperature varies from 12.8°C to 23.4°C during the period of investigation. A total 427.8 mm rainfall was received during the period of investigation which was 176.5 mm in the month of July and 27.5 mm in December, 2019. The soil of the experimental field has a pH of 6.08 (Jackson 1967), with 1.55 Kg ha⁻¹ soil organic carbon (Walkley and Black 1934), 141.12 Kg ha⁻¹ available Nitrogen (Subbianh and Asija 1956), 19.65 Kg ha⁻¹ available Phosphorous (Olsen et al 1954) and 112 Kg ha⁻¹ available Potassium (Stanford and English 1949).

Plant materials and treatment details: One year old seedlings of Celtis australis raised in polybags having uniform shoot length were selected for the experiment. The fields were divided in the experimental plot size of 2 m × 2.4 m. The experiment was laid out in Randomized Block Design (RBD) with three replications having 20 seedlings per plot. The roots were pruned at 0 cm (L_1), 6 cm (L_2) and 12 cm (L_3) length from the collar region and four doses of nitrogen i.e. 0 Kg N ha⁻¹ (N₁), 50 Kg N ha⁻¹ (N₂), 100 Kg N ha⁻¹ (N₃) and 150 Kg N ha⁻¹ (N₄) were applied. The nitrogen was applied in the form of CAN (Calcium ammonium nitrate) with 25% of available nitrogen in two split doses, half at the time of planting and another half after fifteen days of planting. The seedlings were transplanted to the experimental plots on the onset of monsoon i.e. in mid July with 50 cm spacing between plants and 48 cm between rows. The plots were properly managed with periodic hand weeding and irrigation as and when required.

Observations recorded: The observations on growth, nutritional content and physiological parameters were recorded at 150 days after planting (DAP), except for chlorophyll content which was recorded at 120 DAP. Growth parameters viz. shoot length (cm), root length (cm), collar diameter (cm), number of leaves per plant and dry weight of shoot, root and leaves (g plant⁻¹) were recorded. For dry weight, the plant portions were dried in oven at 104°C for 72 hours (or till sample obtained constant weight). In physiological and nutritional parameters: white root regeneration, survival percentage (%), NPK concentration (%) and uptake (Kg ha⁻¹) and chlorophyll content (mg g⁻¹) were determined. The data was subjected to statistical analysis using randomized block design using OPSTAT software.

Image Image <th< th=""><th></th><th>Shot</th><th>Shoot length (cm)</th><th>(cm)</th><th></th><th></th><th>Roo</th><th>Root length (cm)</th><th>(cm)</th><th></th><th></th><th>Number</th><th>Number of leaves plant-1</th><th>plant-1</th><th></th><th></th><th>Collar</th><th>Collar diameter (cm)</th><th>(cm)</th><th></th></th<>		Shot	Shoot length (cm)	(cm)			Roo	Root length (cm)	(cm)			Number	Number of leaves plant-1	plant-1			Collar	Collar diameter (cm)	(cm)	
	۲	N2	N3	N3 N4 Mean	Mean	N1	N2	N3	N4	Mean	N1	N2	N3	N4	Mean	N	N2	N3	N4	Mean
L1	79.28	79.28 83.68	89.64	89.64 85.40 84.50	84.50	26.84	27.53	31.18	30.54	29.02		63.00 95.00 134.00 85.00 94.25	134.00	85.00	94.25	0.81	0.85	1.21	0.96	0.96
L2	87.23	88.38	95.01	90.77	90.34	28.82	30.21	35.91	35.26	32.55		79.83 162.33	99.66	96.66 101.83	110.16	0.95	1.24	1.22	1.37	1.20
L3	86.52	88.23	95.17	92.94	90.72	36.00	35.26	42.40	38.97	38.16	81.83	114.00	142.16	147.66	114.00 142.16 147.66 121.41	1.26	1.37	1.46	1.36	1.36
Mean	84.34	86.76	93.27	89.70		30.55	31.00	36.50	34.92		74.88	123.77	123.77 124.27 111.50	111.50		1.01	1.15	1.30	1.23	
CD	_	z	L ×N			_	z	L×N			_	z	L×N			_	z	L×N		
(b=0.05)	5.31	6.13	S/N			2.43	2.81	N/S			17.73	17.73 20.47	35.46			0.04	0.04	0.08		

RESULTS AND DISCUSSION

Shoot length, root length and number of leaves: Shoot length, root length and number of leaves per plant were significantly (p<0.05) influenced by root pruning and nitrogen application. In case of root pruning, the maximum shoot length (90.72 cm), root length (38.16 cm) and number of leaves (121.41) were recorded in 12 cm root pruned seedlings while among nitrogen doses, the maximum length of shoot (93.27 cm), root (36.50 cm) and maximum number of leaves (124.27) were obtained in 100 Kg N ha⁻¹. The interaction effect between length of root pruning and nitrogen doses were found to be statistically non-significant for shoot and root length while significant (p<0.05) for number of leaves per plant (Table 1). The increase in root pruning lengths showed increase in shoot length due to increase in number of root branching which might have produced high growth-promoting substance which were transferred to the shoot portion. Increase in root length might be due to the availability of water in the soil which increase new root hairs growth, causing absorption of water and nutrients more effectively and also due to the accumulation of auxin on root tips which helps to increase the length of roots. Higher number of leaves per plant might be due to increase in auxin and also its effect on the process of photosynthesis. The similar findings were noted by Sung-Joon et al (2013). Increase in these parameters due to nitrogen application might be due to the increase in cytokinin production which subsequently improves cell growth resulting in higher shoot length and number of leaves per plant whereas, nitrogen fertilizer affects water use efficiency by influencing root growth and enhances nutrient and water acquisition from the soil which helps to increase the root length. These results were similar with the findings of Yang and Fan (2012) and Nigatu et al (2019).

Collar diameter: The root pruning and nitrogen application also had a significant (p<0.05) effect on the collar diameter. Among the root pruning treatments, the maximum (1.36 cm) value for collar diameter was recorded in 12 cm root pruned seedlings. The reason may be that the reserve

carbohydrates may have been used for the cambial growth and is also influenced by the photosynthesis process. The similar results are reported by Kerketta et al (2017) and Kowalska and Kasprzyk (2018). Whereas, among the nitrogen doses, the highest (1.30 cm) value for collar diameter was reported in N @100 Kg ha⁻¹ which might be due to the increase in cytokinin production, which subsequently affects cell wall elasticity, number of meristematic cells and cell growth and increases the root collar diameter. The similar results were obtained in the experiments of Sun et al (2010) and Andivia et al (2011). The interaction effect was also found to be statistically significant (p<0.05) (Table 1).

Dry weight of shoot, root and leaves: The dry weight of shoot, root and leaves increases with increasing root pruning length and nitrogen doses and shows significant (p<0.05) results among the treatments. For root pruning length, significantly highest dry weight of shoot, root and leaves were recorded in L₃(12cm root pruned seedlings) i.e., 8.35 g, 7.64 g and 5.08 g respectively. For nitrogen application, significantly highest dry weight of shoot, root and leaves was observed in N_3 (100 Kg ha⁻¹) i.e., 8.39 g, 7.38 g and 5.08 g respectively. Interaction effect of both root pruning and nitrogen doses was also found to be statistically significant (p<0.05) (Table 2). This might be due to the increase in auxin production in root pruned seedlings (Miller and Graves 2019) which increases growth parameters (shoot length, root length, collar diameter and number of leaves per plant). The increase in carbohydrates production, proteins synthesis and other organic compounds might have increased the growth of plants which may be responsible for increase in shoot, root and leaves biomass of seedlings. These results are supported by the findings of Luna et al (2014) and Zhang et al (2017).

White root regeneration: The white root regeneration has been significantly (p<0.05) affected by both root pruning and nitrogen application. With increase in nitrogen level, root regeneration enhanced and was found maximum in N_3 (22.55). In case of root pruning white root regeneration also increased with increase in length of root pruning and was

Table 2. The effect of root pruning length and nitrogen application on dry weight of shoot, root and leaves in *Celtis australis* seedlings

		Dry we	eight of sl	hoot (g)			Dry we	eight of sh	ioot (g)			Dry we	ight of lea	aves (g)	
	N1	N2	N3	N4	Mean	N1	N2	N3	N4	Mean	N1	N2	N3	N4	Mean
L1	6.82	7.06	8.29	6.96	7.28	5.78	6.82	6.20	6.56	6.34	3.36	3.07	4.79	5.80	4.25
L2	7.09	7.06	8.50	6.89	7.39	7.26	7.26	7.00	7.86	7.34	4.79	4.82	5.50	3.37	4.62
L3	7.39	8.40	8.39	9.20	8.35	6.10	7.86	8.95	7.64	7.64	4.51	5.51	4.84	5.45	5.08
Mean	7.10	7.51	8.39	7.68		6.38	7.31	7.38	7.35		4.22	4.47	5.04	4.87	
CD	L	Ν	L×N			L	Ν	L×N			L	Ν	L×N		
(p=0.05)	0.52	0.60	1.05			0.48	0.55	0.96			0.44	0.51	0.88		

found maximum (25.91) in L_3 (root pruned at 12 cm from collar region). The interaction effect was also found to be statistically significant (p<0.05) (Table 3). The supply of fertilizers might have contributed to influence the root stimulation of seedlings and the process of root initiation in plants. The increase in auxin and cytokinin concentration in root xylem sap after 24 hours of root pruning has been reported by Carlson and Larson (1977), which might have increased the root regeneration in seedlings. This is in line with the findings of Nambiar et al (1979) in *Pinus radiata* and douglas fir. The age and physiological status of the plant also have an influence on white root regeneration, the younger the plant the higher the root regeneration potential (Geisler and Ferree 1984). The similar finding was observed by Vasishth et al (2007).

Survival percent (%): Root pruning and nitrogen application also revealed significant (p<0.05) effect on the survival percent of C. australis seedlings. The survival percent was improved with the application of nitrogen and the highest percent (94.66%) was observed with 100kg N/ha (N₃) and root pruned seedlings at 12cm from collar region (L₃) treatment recorded the maximum (94.50%) survival percent among the root pruning treatments. The interaction of both factors, also revealed significant (p<0.05) variations among the various treatments (Table 3). Addition of nitrogen fertilizer increased the seedling length; the larger seedlings generally display a greater photosynthetic rate and have a higher net carbon gain which may increase the survival rate. The root growth and fibrous root development improves the root surface area and root-soil contact required for adequate water and nutrient absorption for plant growth thus, avoiding planting stress for ensuring plant survival. Similar observations were made by Grossnickle (2005) and Luis et al (2009).

Chlorophyll 'a', 'b' and total chlorophyll (mg g⁻¹): The perusal of data for chlorophyll 'a', 'b' and total chlorophyll revealed significant (p<0.05) effect of root pruning and nitrogen level. The data revealed that the root pruning length of 12 cm (L₃) had significant effect on Chlorophyll 'a' 'b' and total chlorophyll, whereas the maximum concentration was recorded in L₃i.e., 2.48 mg g⁻¹, 13.84mg g⁻¹ and 12.19mg g⁻¹ respectively. This might be due to residual effects which produced the highest significant content of chlorophyll (Yehia et al 2014) and also due to the higher sucrose content present in the seedlings (Tognetti et al 2013). Among the nitrogen doses, maximum concentration of chlorophyll 'a', 'b' and total chlorophyll, was obtained under N₃i.e., 3.41mg g⁻¹, 12.62 mg g⁻¹ and 11.11 mg g⁻¹ respectively, which might be due to the addition of nitrogen which promotes the formation of active photosynthetic pigments by increasing the amount of

White root regeneration Survival pe	>	White root regeneration	oot reg€	eneratio	ç		Surviv	Survival percent (%)	ent (%)	~		Chloro	; Ilyhqu	Chlorophyll 'a' (mg g')	3_ ¹)		Chloro	'd' llyho	Chlorophyll 'b' (mg g ⁻¹)	<u> </u>	F	Total Chlorophyll (mg g ⁻¹)	orophy	l (mg g	(₁
	۲ ۲	N2	N3	N4	Mean	۶	N1 N2 N3 N4 Mean N1 N2 N3	N3	N4	Mean	۲ ۲	N2	N3	N4	Mean	Ŋ	N2	N3	N4	N4 Mean N1 N2 N3 N4 Mean N1 N2 N3 N4 Mean N1 N2 N3 N4 Mean	Ŋ	N2	N3	N4	Mean
L	12.66	12.33	16.33	16.50	14.45	80.00	75.00	98.00	86.00	84.75	0.84	0.48	1.99	2.85	1.54	9.39	9.56	9.44	11.18	12.66 12.33 16.33 16.50 14.45 80.00 75.00 98.00 86.00 84.75 0.84 0.48 1.99 2.85 1.54 9.39 9.56 9.44 11.18 9.89 9.21 8.75 6.64	9 <u>.</u> 21	8.75	6.64	9.31 8.47	8.47
Г2	16.50	16.50	16.66	16.33	16.50	<u>90</u> .00	94.00	16.50 16.50 16.66 16.33 16.50 90.00 94.00 88.00 94.00 91.50 2.07 2.82 1.58 1.21 1.92	94.00	91.50	2.07	2.82	1.58	1.21	1.92	10.17 9.98 7.50 10.58	9.98	7.50	10.58	9.55		8.30 8.40	8.28	9.91 8.72	8.72
Г3	16.16	26.00	34.66	26.83	25.91	90 [.] 00	16.16 26.00 34.66 26.83 25.91 90.00 94.00 98.	98.00	00 96.00 94.50 0.46 1.63	94.50	0.46	1.63	6.68	6.68 1.17	2.48		11.74	20.92	11.78	10.92 11.74 20.92 11.78 13.84	9.63	10.36 18.43 10.36 12.19	18.43	10.36	12.19
Mean	Mean 15.11 18.27 22.55 19.88	18.27	22.55	19.88		86.66	86.66 87.66 94.6	90	92.00		1.12	1.12 1.64 3.41 1.74	3.41	1.74		10.16	10.42	10.16 10.42 12.62 11.18	11.18		9.04	9.17 11.11	11.11	9.86	
C	_	L N L×N	L×N			_	L N L×N	Γ×Ν				z	N L×N			_	N L×N	L×N			_	z	L× N		
(0.05)	2.289	2 289 2 643 4 579	4.579			1.70	1.70 1.96 3.4	3.40			0.30	0.30 0.34 0.60	09.0			1.47	1.47 1.70 2.95	2.95			0.88	0.88 1.02 1.77	1.77		

Table 4. The effect of root pruning length and nitrogen application on NPK concentration in Celtis australis seedlings

-		Nitrogen	i concent	ration (%	%)	Pho	osphorou	us concei	ntration	(%)	Po	otassium	n concent	ration (%	%)
	N1	N2	N3	N4	Mean	N1	N2	N3	N4	Mean	N1	N2	N3	N4	Mean
L1	0.30	0.01	0.28	0.01	0.15	0.22	0.28	0.24	0.32	0.26	0.25	0.50	1.25	0.50	0.62
L2	0.01	0.48	0.31	0.30	0.27	0.28	0.28	0.32	0.26	0.28	0.50	0.24	1.50	0.50	0.68
L3	0.87	0.78	1.04	1.29	0.99	0.23	0.26	0.39	0.30	0.29	1.00	1.25	1.25	1.50	1.25
Mean	0.39	0.42	0.54	0.53		0.24	0.27	0.31	0.29		0.58	0.66	1.33	0.83	
CD	L	Ν	L×N			L	Ν	L×N			L	Ν	L×N		
(p=0.05)	0.01	0.02	0.03			0.01	0.02	0.03			0.23	0.26	0.46		

Table 5. The effect of root pruning length and nitrogen application on NPK uptake in Celtis australis seedlings

		Nitroge	en uptake	e (kg ha¹)	P	hosphore	ous upta	ke (kg ha	1 ⁻¹)		Potassiu	m uptak	e (kg ha⁻¹)
	N1	N2	N3	N4	Mean	N1	N2	N3	N4	Mean	N1	N2	N3	N4	Mean
^L 1	0.71	16.43	0.18	17.56	8.72	12.65	7.82	14.37	15.66	12.62	27.68	12.88	34.52	27.81	25.72
^L 2	26.40	0.70	16.95	7.13	12.79	15.00	16.31	4.28	15.00	12.64	13.23	26.97	78.40	6.77	31.34
[⊾] 3	14.09	42.60	101.21	64.44	55.58	4.15	18.59	30.60	12.73	16.51	18.06	61.20	77.45	116.35	68.26
Mean	13.73	19.91	39.44	29.71		10.60	14.24	16.41	14.46		19.65	33.68	63.45	50.31	
CD (0.05)) L	Ν	L×N			L	Ν	L×N			L	Ν	L×N		
	0.01	0.01	0.02			1.75	2.02	3.50			0.37	0.43	0.75		

stromal and thylakoid proteins in leaves (Filho et al 2011). Chlorophyll and carotenoid synthesis are also dependent upon mineral nutrition (Daughtry and Mcmurtey 2000). The interaction effect for chlorophyll 'a', 'b' and total chlorophyll between length of root pruning and nitrogen doses was found to be statistically significant (p<0.05) (Table 3).

Nutrients content (%) and uptake (Kg/ha): Root pruning and nitrogen application had significant (p<0.05) effect on NPK content and uptake. Among L₃ root pruning length, the maximum NPK content of 0.99%, 0.29% and 1.25% were recorded and similarlythe highest NPK uptake of 55.58 Kg ha ¹, 16.51 Kg ha⁻¹ and 68.26 Kg ha⁻¹ were obtained. This might be due to the absorption capacity of roots, mass flow and diffusions of roots. As the root system regenerates, uptake may increase accordingly. The efficiency of roots in uptake of nutrients depends on the amount of surface in contact with soil and on the permeability of root surface. Root regeneration after root pruning provides more root branches which increases the absorbing surface. These factors suggest that uptake of nutrients will be the same or even improved when the root system is regenerated. The decrease in concentration and uptake of nutrients (NPK) in severely pruned seedling (6 cm) may be due to greater decrease in root biomass which has affected the absorption capacity of root, which could have decreased xylem functioning, poor conductivity of the soil surrounding the roots, increase in the content resistance between soil and roots (Dhiman 1991). The similar findings were reported by Vasishth et al (2007) in Acacia catechu. Among the nitrogen application, the maximum concentration and uptake of NPK were recorded in N₃i.e., 0.54%, 39.44 Kg ha⁻¹, 0.31%, 16.41 Kg ha⁻¹ and 1.33%, 63.45 Kg ha⁻¹. With increasing nitrogen application, nitrogen content and uptake has increased in the seedlings up to N3 level (100kg N/ha) and decreased afterwards. Nitrogen content in plants increased due to the good availability of applied fertilizer for the whole growing period (Kaplan et al 2015). Increase in concentration of highly mobile elements such as P and K in stressed plants could be partly due to their absorption throughout the seasons (Mead 1984). The similar findings were also reported by Fang et al (2017) and Mensah et al (2020). The interaction effect for NPK uptake between length of root pruning and nitrogen doses was also found to be statistically significant at 150 days after planting (p<0.05) (Table 4 & 5).

CONCLUSIONS

On the basis of results of the present study, it can be concluded that root pruning and nitrogen application enhanced the growth performance and physiological characteristics of *Celtis australis* seedlings. Root pruned at 12 cm from collar region and application of nitrogen @ 100 Kg ha⁻¹ were considered best for the growth performance and establishment of the seedlings. Nitrogen application and root pruning combinations recovered the plants from the stress condition and enhanced the morphological as well as physiological characteristics of seedlings.

AUTHOR CONTRIBUTIONS

Conceptualization, P.U. and A.V.; methodology, A.V., B.S., D.R. and I.S.; software, P.U., D.R. and S.T.; investigation, P.U.; writing-original draft preparation, P.U. and S.T.; writing-review and editing, P.U., A.V., B.S., D.R. and I.S.;

supervision, A.V., B.S., D.R. and I.S.

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