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RESEARCH NOTE

Isolation of β-glucosidase from different parts of maize (*Zea mays*) and its specific activity estimation

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β-glucosidase is a remarkable enzyme in the field of enzyme technology. Firstly, it helps in producing ethanol from the plant waste and subsequently helps to cope up the oil crisis as well as in alcohol based industries. Secondly, it improves the taste and aroma of wine, tea and fruits. In the present study β-glucosidase was isolated from different plant parts of maize (*Zea mays*) and specific activity was estimated. The maximum crude enzyme was found in coleoptile (2.86ml) and maximum specific activity was estimated in thumb leaf (lower most leaf) as 43.446 whereas maximum protein was found in second leaf blade (9.241 mg/ml).

Key words : Maize, β-glucosidase, Specific activity, Protein

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β-glucosidases (β-D-glucoside glucohydrolases; EC 3.2.1.21) occur ubiquitously in plants, fungi, mammals and micro-organisms (Woodward and Wiseman, 1982). β-glucosidases have been the subject of much recent research due to the key role these enzymes play in biological processes and potential biotechnological applications. Among them, plant β-glucosidases play role in defense against pests (Bell, 1981; Niemeyer, 1988; Poulton, 1990), phytohormone activation (Brzobohaty *et al.*, 1990; Matsuzaki and Koiwai, 1986; Schliemann, 1984; Smith and van Staden, 1978; Wiese and Grambow, 1986), lignifications (Dharmawardhana, 1995) and cell wall catabolism (Leah *et al.*, 1995; Simos *et al.*, 1994). The present investigation was carried out to extract β-glucosidase from different parts of maize plant and its specific activity estimation.

Extraction of crude enzyme from maize plant :

Maize (*Zea mays* L.) β-glucosidase (β-d-glucosideglucohydrolase, EC 3.2.1.21) was extracted from the different parts of maize plant such as coleoptile, radicle, node, mesocotyl, thumb leaf (lower most leaf), flag leaf (top most leaf below tassel), first leaf blade and second leaf blade, of 10 to 15 days -old maize seedlings with 50 millimolar sodium acetate, pH 5.0. The pH of the extract was adjusted to 4.6, and most of the contaminating proteins were cryoprecipitated at 0°C for 24 hours. The pH 4.6 supernatant from cryoprecipitation will be further fractionated for enzyme activity and total soluble protein estimation.

Plant material :

Maize (*Zea mays)* seeds were obtained from the local market of Allahabad. Seeds were geminated in vermiculite in darkness (4-6 days) and light (5-7) days at 25^0C. Seedlings were harvested and divided into different parts for enzyme assays. The seedlings were divided into the different parts *viz.*, the coleoptiles, radicle, node, mesocotyl, thumb leaf (lower most leaf), flag leaf (top most leaf below

tassel) first leaf blade, and second leaf blade.

-Glucosidase assay :

The different plant parts of maize were grounded to fine powder in a chilled mortar and pestle using liquid nitrogen and extraction buffer (1 ml buffer/ 1 g tissue) was mixed to it. This was centrifuged at 12000 rpm / 4^0C / 30 min. The supernatant was taken and it was used for activity and protein estimation. For activity estimation, paranitophenyl-β-D-glucopyranoside (pNPG) was used as a substrate. Optical density was taken using spectrophotometer at 405 nm for experimental and control reaction. For experimental, 165 µl citrate phosphate buffer (0.1 M, pH 4.8), 10 µl crude enzyme and 25 µl substrate (pNPG) were mixed at room temperature. After 15 min 800 µl, 1 M Na_2CO_3 was mixed to stop the reaction and O.D. was taken. For control, 165µl citrate phosphate buffer (0.1 M, pH 4.8), 10 µl enzyme, 800 µl Na_2CO_3 (1M) and 25µl substrate (pNPG) were mixed at room temperature and after 15 min, O.D. was taken (Mahadaven and Sridhar, 1986).

The enzyme from each part of maize was extracted with procedure described by Mahadaven and Sridhar (1986). The volume of crude enzyme and weight of respective tissues taken for various parts of the plant are tabulated in Table 1.

Table 1 : Volume of crude enzyme of maize

Sr. No.	Sample	Wt. of tissue taken (g)	Volume of crude enzyme (ml)
1.	Coleoptile	2.812	2.86
2.	Radicle	1.421	2.62
3.	Node	0.563	0.35
4.	Mesocotyl	0.052	0.32
5.	Thumb leaf (lower most leaf)	1.412	1.50
6.	Flag leaf (top most leaf below tassel)	0.621	0.91
7.	First leaf blade	1.323	0.54
8.	Second leaf blade	1.411	0.65

Table 2 : -glucosidase activity profile from various parts of the plant in maize

Sr. No.	Sample	Change in O.D./10µl	Activity units/ml/min.	Activity (%)
1.	Coleoptile	0.061	400	23.5
2.	Radicle	0.264	1760	100
3.	Node	0.031	206	11.17
4.	Mesocotyl	0.164	1093	62.1
5.	Thumb leaf (lower most leaf)	0.075	500	28.40
6.	Flag leaf (top most leaf below tassel)	0.094	627	35.625
7.	First leaf blade	0.25	1667	94.72
8.	Second leaf blade	0.132	880	50.0

Table 3 : Protein estimation in maize

Sr. No.	Sample	Protein mg/ml	Protein mg/g	Specific activity
1.	Coleoptile	2.148	1.6703	21.41
2.	Radicle	3.497	2.4636	10.008
3.	Node	3.584	2.5719	3.499
4.	Mesocotyl	1.581	0.4165	8.855
5.	Thumb leaf (lower most leaf)	1.381	1.2118	43.446
6.	Flag leaf (top most leaf below tassel)	1.612	0.7988	6.8823
7.	First leaf blade	9.216	6.2976	6.986
8.	Second leaf blade	9.241	7.8995	6.076

Activity estimation of maize :

The activity of crude enzyme in maize per gram fresh weight basis was highest in radicle (100%) and followed by first leaf blade (94.715%). Mesocotyl (62.1%) had also high activity while lowest in node (11.17%) as shown in Table 2.

Total soluble protein and specific activity estimation from extracts of maize :

From protein estimation data, it was noticed that first leaf blade and second leaf blade had highest protein 6.2976 mg and 7.8995 mg, respectively and lowest protein content was found in mesocotyl (0.4165 mg) of maize. The specific activity was highest in thumb leaf (43.446) as shown in Table 3.

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