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Seed protein profiles in Celery (*Apium graveolens* L.) and Ajowan (*Trachyospermum amni* L.) plant types

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SUMMARY

Electrophoretic banding patterns of seed protein following SDS-PAGE in celery (*Apium graveolens* L.) and ajowan (*Trachyospermum amni* L.) plant types (controls and 10 induced macromutants in each species) demonstrated gross similarities and differences among the plant types of the species in relation to number of bands (celery : 8-15 bands, total 18 types; ajowan : 9-20 bands, total 21 types), molecular weights (celery : 84.2 kD – 14.6 kD; ajowan : 76.8 kD – 15.0 kD), band percentage (celery : 0.2 – 28.6; ajowan : 2.7 – 10.3) and pixel intensity. Protein profiling revealed the presence of specific marker bands in few genotypes of the species.

Key words : Celery, Ajowan, Macromutants, SDS-PAGE, Protein profiles.

Protein profiling from electrophoretic banding patterns of seed protein have been used effectively to ascertain interrelationship between genotypes and screening reliable protein markers (Bannet *et al.*, 1991, Cook 1984, Datta and Saha 2003, Kumar and Ram 1989, Sengupta *et al.*, 2004). With a view to it, present investigation has been undertaken to characterize the plant types (control and 10 induced macromutants) of celery (*Apium graveolens* L.) and ajowan (*Trachyospermum amni* L.) following electrophoretic banding (SDS-PAGE) polymorphism. Celery and ajowan are members of Apiaceae and yields spice of commerce.

MATERIALS AND METHODS

To study protein polymorphism in celery and ajowan (control – C; mutants : *thick stem* I and II – TS I and II, *pigmented stem* – PS, *lax branching* I and II – LB I and II, *bushy* – Bu, *dwarf*- Dw, *early flowering* – EF, *late flowering* – LF, *funnel* – Fu, *drooping branched* – DB, *broad pinnae* – BP and *narrow pinnae* – NP) plant types, one dimensional SDS-PAGE (10.0% separating gel and 4.5% stacking gel) was carried out following Laemmli (Laemmli 1970) in a vertical gel system (BIOTECH). For the purpose, total protein was extracted in 0.2 M Tris-HCl buffer (pH – 8.5), suspended overnight (0 - 4^{0}C) and centrifuged at 15,000 rpm (- 4^{0}C) for 30 minutes (3-4 times). The protein samples along with sample buffer containing bromophenol blue were hydrolyzed in boiling water (1-2 mins.), cooled and loaded in lanes with micropipette (8 µl/lane). A protein molecular weight marker (GENEI Bangalore, Cat No. PMW-M) was also incorporated into the gel (as marker lane) as reference to detect molecular weights of the bands. The gel was run at 36 mA (3 mA/lane) for 2-3 hours, stained in Coomassie Brilliant Blue R 250 for overnight, destained and stored in 7% acetic acid. Gel preparations were analyzed in a gel documentation unit (Ultra Lum, USA) using the software Total Lab. Band were detected and molecular weights, band percentage (thickness of the bands) and pixel peak (based on area, volume and intensity of the bands) of each band were computed. Bands were classified based on molecular weights (very high : > 70.0 kD, high : 70.0 kD – 40.0 kD, medium : 39.0 kD – 25.0 kD and low : < 25.0 kD) as well as band intensity (celery : - intensed : > 25.0, medium : 25.0 – 10.0 and faint : < 10.0; ajowan :- intensed : > 115.0, medium : 115.0 – 100.0 and faint : < 100.0).

RESULTS AND DISCUSSION

Celery (Table 1, Fig. 1): A total of 18 polypeptide bands (Rm value : 0.257 – 0.956) of diverse molecular weights ranging from 84.2 kD to 14.6 kD have been noted in 11 (control and 10 macromutants) plant types, and the band percentage of those bands ranged from 0.2 (band no. 10 and 15) to 28.6 (no. 3). Apart from polypeptide band 3 (28.6%), band no. 8 (22.7%), 17 (15.2%), 9 (13.0%) and 6 (11.4%) were also prominent bands. Number of bands varied (Dw-8; Bu-9; Fu-10; LB- I and BP-12; PS-13; C, TS I, NP, EF and LF-15) among the plant types. Mostly the polypeptide bands were of medium (4 to 7) to low (2 to 6) molecular weights. Mutants had 1 very high and 1 high molecular weight bands; while, control showed 3 high molecular weight bands. Based on pixel intensity, polypeptide bands have been intensed (0 to 6), medium (2 to 6)and faint (2 to 9) among the plant types. Band no. 1, 3, 6, 7, 8, 9, 17 and 18 were uniformly present in all plant types; while, 2, 4, 5 have been documented only in control. Polypeptide band numbers 10, 15 and 16 (C, Fu, Bu, and Dw) failed to express in few plant types. Band 15 was also absent in *broad pinnae* mutant.

Ajowan (Table 2, Fig. 2): A total of 21 polypeptide bands (Rm value : 0.277 to 0.979) of diverse molecular weights (76.8 kD to 15.0 kD) have been noted in the plant

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