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(Short Communication)

Polyamine levels in anthers of poly-cytoplasmic isonuclear male sterile lines of pearl millet

Rewa Dhillon-Grewal¹, D. S. Virk², B. K. Mangat², R. K. Basra¹ and A. S. Basra^{1,*}¹Department of Botany and ²Plant Breeding, Punjab Agricultural University, Ludhiana-141 004, India

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Abstract. This work is the first investigation of polyamine levels in anthers of isonuclear lines of 81B maintainer line of pearl millet (*Pennisetum typhoides*) in four cytoplasms. Cytoplasmic specificity in polyamine levels was detectable among different sterile cytoplasms and sterile versus fertile cytoplasms but not amongst the three polyamines, viz. putrescine, spermidine and spermine. The different levels of polyamines are attributable to the cytoplasm. Control of polyamine synthesis by cytoplasm is indicated.

Key words: Anthers; Cytoplasmic male sterility; Isonuclear lines; Pearl millet; *Pennisetum typhoides*; Polyamines.

Introduction

The diamine putrescine and polyamines spermidine and spermine are widely distributed in plants, and changes in polyamine levels and their biosynthetic enzymes have been correlated with several growth and developmental processes (Evans and Malmberg, 1989; Galston and Sawhney, 1990). They occur in the free forms as cations, but often are conjugated to small molecules such as phenolic acids as well as to various macromolecules. The expression of male sterility has also been associated with changes in endogenous levels of polyamines (Martin-Tanguy *et al.*, 1979, 1982). Recently, studies by Rastogi and Sawhney (1990a, b) have suggested that the elevated polyamine levels contribute to abnormal stamen development in a male-sterile mutant of tomato. However, no information is available on polyamine levels in poly-cytoplasmic isonuclear male sterile lines of any plant to validate their involvement.

*Corresponding author.

Cytoplasmic genetic male sterility is being commercially exploited in the single cross hybrid breeding programmes in pearl millet. All pearl millet hybrids so far have been exclusively bred on A₁ (Tift 23A) source of cytoplasm but cyclic appearance of downy mildew on A₁ based hybrids has necessitated assessment of genomic and cytoplasmic diversification of male sterile parents. Three additional sources of cytoplasm, A₂ and A₃ (Burton and Athwal, 1967) and *violaceum* (Marchais and Pernes, 1985) are now available which have shown stable and exploitable sterility systems. But before cytoplasmic diversification is exploited commercially, it is imperative to determine if the various cytoplasms differ. The objective of this research was, therefore, to study cytoplasmic differentiation with respect to polyamine levels in isonuclear lines with variable cytoplasms.

Materials and Methods

The study on the estimation of three polyamines, i. e. putrescine, spermidine and spermine was conducted by developing isonuclear lines of 81B maintainer line of

International Crops Research Institute for the Semi-arid Tropics (ICRISAT, Hyderabad) in four cytoplasms. The male sterile line 81A (=ICMA-1) was developed at ICRISAT by irradiating Tift 23 DB followed by successive back-crossing which resulted in the pair 81A (male sterile) and 81B (maintainer) lines. This line in A_1 source has been extensively used in India for development of new hybrids as it possesses good combining ability. Therefore, the line 81B was selected for genomic substitution into other cytoplasms. The 81B genome was substituted into *violaceum* cytoplasm (Marchais and Pernes, 1985) to give a male sterile line 88001A at ICRISAT. The 81B genome was simultaneously substituted into the A_2 and A_3 cytoplasms at the Punjab Agricultural University, Ludhiana by successive back-crossing of 81B line on to Pb 305 A_2 and Pb 405 A_3 male sterile lines. This has resulted in Pb 310 A_2 and Pb 406 A_3 isonuclear lines of 81A. Thus 81 A_1 , 88001A (ν) 81A (ν) Pb 310 A_2 (81 A_2) and Pb 406 A_3 (81 A_3) isonuclear lines with the same genome but cytoplasmic variation along with the corresponding maintainer line 81B carrying the fertile cytoplasm were used in the present study.

The material consisting of 4 male sterile and 1 male fertile isonuclear lines was raised in the field. Five inflorescences in each line were bagged before emergence from the flag leaf. The inflorescences were collected when the anthers appeared in the bag. The anthers were collected for polyamine estimation.

Extraction and determination of polyamines was performed according to Szczotka (1984). Anthers were extracted in 5% (v/v) cold $HClO_4$ at a ratio of about 100 mg/ml $HClO_4$ and centrifuged for 15 min at 9,000 g. The sediments were suspended, centrifuged and the supernatants pooled and transferred to Dowex-50 WX-4 (200-400 mesh) columns (90 × 4 mm). The columns were washed with 40 ml of 0.1 M sodium-phosphate

buffer (pH 8.0 in 0.7 M HCl) followed by 10 ml of 1N HCl. The polyamines were eluted with increased concentration of 5 ml HCl: putrescine 2M HCl, spermidine 3M HCl and spermine 4M HCl. After evaporation of eluates to dryness, each sample was dissolved in 0.5 ml of water and portions of 100 μ l were put onto filter paper, dried and sprayed with ninhydrin reagent comprising of 1.0 g ninhydrin, 0.1 g cadmium acetate, 10 ml water, 5 ml acetic acid and 85 ml acetone. The spots became red after heating at 70°C for 60 min. Spots were extracted with 3 ml of solvent consisting of 0.2 g cadmium acetate, 10 ml water, 40 ml ethanol, and 50 ml acetic acid. Extraction was done at room temperature for 30 min, and absorbance was measured at 505 nm. A comparative curve was plotted with the help of standard polyamines passed previously through the Dowex 50 column.

An analysis of variance was performed with polyamines and cytoplasms as main effects. The mean squares for the main effects were tested against polyamines \times cytoplasms mean squares (σ_e^2) and the least significant difference (LSD) was calculated as $\sqrt{2\sigma_e^2/3} \times t_{\alpha}$ d.f. at $\alpha=0.05$ and 0.10 for comparing the cytoplasms.

Results and Discussion

The estimates of putrescine, spermidine and spermine are given in Table 1. Cytoplasmic specificity in polyamine levels was observed among different sterile cytoplasms and sterile vs fertile cytoplasms. The analysis of variance (Table 2) revealed significant differences among the cytoplasms but not among the three polyamines.

The examination of mean values of polyamines for cytoplasms revealed that at $\alpha=0.05$ the A_3 (81 A_3 or Pb 406 A_3 line) cytoplasm had significantly lower estimate

Table 1. Endogenous levels of polyamines (μ moles/g F.W.) in five cytoplasms of pearl millet with the same genome

Cytoplasm	Putrescine	Spermidine	Spermine	Mean
81 B (fertile)	0.715	1.4297	2.300	1.482
81 A_1 (A_1 sterile)	2.130	1.1088	4.040	2.426
81 A_2 (=Pb305 A_2 , A_2 sterile)	1.678	1.9180	1.810	1.802
81 A_3 (=Pb406 A_3 , A_3 sterile)	0.430	0.1640	0.795	0.463
81 A (ν) (=88001 A, <i>violaceum</i> sterile)	1.123	0.7800	1.050	0.984
lsd cytoplasms ($\alpha=0.05$)	1.249			
lsd cytoplasms ($\alpha=0.10$)	1.008			

Table 2. Analysis of variance for polyamines and cytoplasms in pearl millet

Source	d.f.	Mean squares	F
Cytoplasms	4	1.7005	3.86*
Polyamines	2	1.2308	2.80
Cytoplasms × Polyamines	8	0.4401	

*Significant at 5% probability level.

than the A₁ (81 A₁) and A₂ or Pb 310 A₂) cytoplasms. The A₁ cytoplasm (81 A₁) had significantly higher polyamines content than the *violaceum* (81 A(v) or 88001 A) cytoplasm. When the $\alpha = 0.10$ probability level was used the A₃ cytoplasm (81 A₃ or Pb 406 A₃) had significantly lower content of mean polyamines than the A₁ (81 A₁), A₂ (81 A₂ or Pb 305 A₂) and fertile (81 B) cytoplasms but was on a par with the *violaceum* (81 A(v) or 88001 A) cytoplasm. The *violaceum* cytoplasms in turn had significantly lower polyamine content than the A₁ (81 A₁) cytoplasm.

Rastogi and Sawhney (1990b) reported that floral organs of a male sterile stamenless - 2 mutant of tomato contain significantly higher levels of putrescine, spermidine and spermine, than the normal. The analysis of polyamine conjugates in the male sterile lines provides another evidence for the possible involvement of polyamines in stamen development. Hydroxycinnamic acid amides occurred in large amounts in the anthers of fertile plants of maize and were absent from the anthers of cytoplasmic male sterile lines (Martin-Tanguy *et al.*, 1979, 1982). Since a clear correlation between free polyamine levels and cytoplasmic male sterility was not discernible in the present study, it would be worthwhile to seek a relationship with polyamine conjugates.

It may be concluded that the various cytoplasms were diverse enough to synthesize different amounts of polyamines which not only establishes the fact that the various cytoplasms differ and either of the polyamines

may be analyzed for discerning cytoplasmic differentiation. Since the cytoplasmic sources differ with respect to the three polyamines studied they provide a good base for cytoplasmic diversification of male sterile lines in pearl millet and hence in widening the genetic base of female parents of the millet hybrids in the future.

Literature Cited

- Burton, G. W. and D. S. Athwal. 1967. Two additional sources of cytoplasmic male sterility in pearl millet and their relationship to Tift 23A. *Crop Sci.* 7: 209-211.
- Evans, P. T. and R. L. Malmberg. 1989. Do polyamines have roles in plant development? *Annual Rev. Plant Physiol. Plant Mol. Biol.* 40: 235-269.
- Galston, A. W. and R. K. Sawhney. 1990. Polyamines in plant physiology. *Plant Physiol.* 94: 406-410.
- Marchais, L. and J. Pernes. 1985. Genetic divergence between wild and cultivated pearl millets (*Pennisetum typhoides*). I Male sterility. *Z. Pflanzenzucht.* 95: 103-112.
- Martin-Tanguy, J., A. Deshayes, E. Perdrizet, and C. Martin. 1979. Hydroxycinnamic acid amides in *Zea mays*: distribution and changes with cytoplasmic male sterility. *FEBS Lett.* 108: 176-178.
- Martin-Tanguy, J., E. Perdrizet, J. Prevost, and C. Martin. 1982. Hydroxycinnamic acid amides in fertile and cytoplasmic male sterile lines of maize. *Phytochemistry* 21: 1939-1945.
- Rastogi, R. and V. K. Sawhney. 1990a. Polyamines and flower development in the male sterile stamenless-2 mutant of tomato (*Lycopersicon esculentum* Mill.). I. Level of polyamines and their biosynthesis in normal and mutant flowers. *Plant Physiol.* 93: 439-445.
- Rastogi, R. and V. K. Sawhney. 1990b. Polyamines and flower development in the male sterile stamenless-2 mutant of tomato (*Lycopersicon esculentum* Mill.). II. Effects of polyamines and their biosynthesis inhibitors on the development of normal and mutant floral buds cultured *in vitro*. *Plant Physiol.* 93: 446-452.
- Szczotka, Z. 1984. Polyamine changes in (*Quercus borealis* Michx.) and (*Quercus robur* L.) seeds during ageing in controlled conditions. *Acta Physiol. Plant.* 6: 127-135.

珍珠黍近同核雄不稔性異細胞質維持系之花藥多聚胺含量

Rewa Dhillon-Grewal¹, D. S. Virk², B.K. Mangat²

R. K. Basra¹ and A. S. Basra¹

¹Department of Botany and ²Plant Breeding

Punjab Agricultural University

Ludhiana-141 004, India

本研究是珍珠黍 (*Pennisetum typhoides*) 81B 細胞質雄不稔性維持系有近同核性 (isonuclear) 之品系在四種不同細胞質下其產生植株花藥的多聚胺 (polyamine) 含量之首次報告。多聚胺含量在細胞質之差異性可在不同的不稔細胞質系及不稔與可稔細胞質系間測出來。但對另外三種多聚胺，腐肉胺 (putrescine) 精胺素 (spermidine) 和精胺 (spermine) 之含量在細胞質內，則無差異性。多聚胺的含量不同可歸因於細胞質之影響，本文結果顯示多聚胺的合成是由細胞質所控制。