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# DIPLOIDIZATION AND FERTILITY IMPROVEMENT IN ADVANCED AUTOTETRAPLOIDS OF *TRIGONELLA FOENUM-GRAECUM*

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## ABSTRACT

**Meiotic analysis of four genotypes of autotetraploid *Trigonella foenum-graecum* over generations ($C_4$, $C_8$ and $C_{12}$ generations) indicated that there is gradual decrease in multivalents and univalents with simultaneous increase in bivalents; thus, revealing diploidization process. Though autotetraploid *Trigonella* is vegetatively vigorous and meiotically regular after 12 years of selection, the seed yield is still < 50% of their diploid progenitors. Therefore, it may be concluded that poor fertility in autotetraploid *T. foenum-graecum* is controlled by certain genetical factors which could only be handled by genetic manipulations.**

Key words: *Trigonella foenum-graecum*, autotetraploid, diploidization, meiotic associations.

Polyploidy has been successfully induced by colchicine in several ornamental, grasses, vegetatively propagated plants, and also in many legumes [1–4]. The literature of past few decades clearly indicates that although cytomorphogenetical studies have been carried out in early generations of induced polyploids and the possibility of evolving more vigorous, high yielding varieties has been indicated, detailed studies on cytomorphological behaviour of highly evolved and stabilized polyploids are almost absent. The present investigation has been carried out on four advanced autotetraploid genotypes of *Trigonella foenum-graecum* to explore the possibility of evolving and stabilizing more vigorous and high yielding varieties of autotetraploids and studying the impact of judicious selections over twelve generations on vigour, fertility and meiotic behaviour.

## MATERIALS AND METHODS

The $C_4$, $C_8$ and $C_{12}$ progenies of four autotetraploid genotypes (Sel. 1, Sel. 2, Sel. 3 and Sel. 4) of *Trigonella foenum-graecum* developed in our laboratory [5] were grown under

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uniform agroclimatic conditions along with their diploid progenitors over different years. The cytomorphological data were collected during their respective generations. Acetocarmine squash preparations were made to study meiosis. Cytomorphology was studied in 20 randomly selected plants of different genotypes at both ploidy levels and selection for the next generation was done accordingly. 400 PMCs were analysed randomly at metaphase I and 50 PMCs at anaphase I in each genotype in $C_4$, $C_8$ and $C_{12}$ generations. Similarly 500 pollen grains from each plant were scored for pollen fertility in each generation.

## RESULTS AND DISCUSSION

Mean values for chiasma frequency, quadrivalents, trivalents, bivalents and univalents frequencies of different genotypes (Sel. 1, Sel. 2, Sel. 3 and Sel. 4) of *T. foenum-graecum* in $C_4$, $C_8$ and $C_{12}$ generations are presented in Table 1. A comparison of mean values of bivalents number and pollen fertility between $C_4$–$C_8$, $C_8$–$C_{12}$ and $C_4$–$C_{12}$ indicated that bivalents differed significantly in all comparisons; pollen fertility differed only in $C_8$–$C_{12}$ and $C_4$–$C_{12}$ but not in $C_4$–$C_8$. Sel. 3 is an exception where $C_8$–$C_{12}$ also did not differ. Thus in Sel. 3, meiotic stabilization is much faster than rest of the three genotypes.

Percentage of equal chromosomal disjunction at anaphase I under succeeding generations (Table 2) increased with advancement of generations. Pollen fertility also increased from $C_4$ to $C_{12}$ generations in all the four genotypes.

**Table 1. Actual mean chiasma frequency and different chromosomal associations in $C_4$, $C_8$ and $C_{12}$ generations of different autotetraploids of *T. foenum-graecum***

| Genotype and generation | | Chiasma frequency per chromosome | Range and frequency per PMC: quadrivalents (IV) range | quadrivalents (IV) mean ± SE | trivalents (III) range | trivalents (III) mean ± SE | bivalents (II) range | bivalents (II) mean ± SE | univalents (I) range | univalents (I) mean ± SE |
|---|---|---|---|---|---|---|---|---|---|---|
| Sel. 1 | $C_4$ | 0.80 ± .02 | 0–3 | 0.69 ± .02 | 0–1 | 0.20 ± .02 | 8–10 | 13.65 ± .08 | 0–4 | 0.80 ± .06 |
| | $C_8$ | 0.78 ± .01 | 0–2 | 0.50 ± .03 | 0–1 | 0.05 ± .01 | 9–16 | 14.26 ± .14 | 0–2 | 0.75 ± .07 |
| | $C_{12}$ | 0.66 ± .01 | 0–2 | 0.15 ± .01 | 0–1 | 0.02 ± .01 | 12–16 | 15.52 ± .06 | 0–2 | 0.23 ± .03 |
| Sel. 2 | $C_4$ | 0.80 ± .01 | 0–3 | 1.20 ± .03 | 0–2 | 0.18 ± .02 | 6–12 | 73.08 ± .06 | 0–4 | 1.26 ± .06 |
| | $C_8$ | 0.78 ± .01 | 0–3 | 0.70 ± .05 | 0–2 | 0.13 ± .05 | 9–14 | 13.55 ± .14 | 0–4 | 0.97 ± .06 |
| | $C_{12}$ | 0.68 ± .01 | 0–2 | 0.07 ± .02 | 0–1 | 0.04 ± .01 | 12–16 | 15.21 ± .06 | 0–2 | 0.47 ± .06 |
| Sel. 3 | $C_4$ | 0.86 ± .01 | 0–3 | 1.08 ± .04 | 0–1 | 0.12 ± .03 | 9–13 | 13.62 ± .13 | 0–4 | 0.93 ± .15 |
| | $C_8$ | 0.85 ± .01 | 0–2 | 0.41 ± .03 | 0–1 | 0.03 ± .01 | 9–16 | 14.33 ± .18 | 0–2 | 0.73 ± .03 |
| | $C_{12}$ | 0.67 ± .01 | 0–1 | 0.18 ± .02 | 0–1 | 0.05 ± .01 | 12–16 | 15.13 ± .06 | 0–2 | 0.43 ± .08 |
| Sel. 4 | $C_4$ | 0.76 ± .02 | 0–2 | 0.85 ± .04 | 0–1 | 0.14 ± .04 | 8–13 | 13.46 ± .29 | 0–4 | 1.07 ± .11 |
| | C | 0.74 ± .01 | 0–2 | 0.29 ± .02 | 0–1 | 0.08 ± .01 | 9–15 | 14.68 ± .13 | 0–2 | 0.44 ± .05 |
| | $C_{12}$ | 0.65 ± .01 | 0–2 | 0.25 ±. 02 | 0–1 | 0.05 ± .01 | 12–16 | 15.03 ± .09 | 0–2 | 0.54 ± .07 |

The morphological parameters of different genotypes in $C_4$ and $C_8$ and $C_{12}$ generations are presented in Table 3. Here the values are presented as percentage of their respective diploid progenitors to get an idea about increase or decrease in relation to diploids. Continuous improvement in the vegetative traits and seed fertility is indicated, but even after 12 years selection the seed yields in different genotypes are still 21%-42% of their diploid progenitors.

**Table 2. Percentage of equal chromosomal disjunction at anaphase I and pollen fertility in different genotypes of autotetraploid *T. foenum-graecum* in $C_4$, $C_8$ and $C_{12}$ generations**

| Genotype | Generation | Equal disjunction (%) | Pollen fertility (%) |
|---|---|---|---|
| Sel. 1 | $C_4$ | — | $81.0 \pm 1.3$ |
| | $C_8$ | $58.3 \pm 2.4$ | $83.5 \pm 0.6$ |
| | $C_{12}$ | $70.5 \pm 1.9$ | $84.2 \pm 1.2$ |
| Sel. 2 | $C_4$ | — | $61.7 \pm 1.2$ |
| | $C_8$ | $52.4 \pm 2.1$ | $69.2 \pm 0.5$ |
| | $C_{12}$ | $61.4 \pm 0.9$ | $77.6 \pm 1.7$ |
| Sel. 3 | $C_4$ | — | $79.6 \pm 1.9$ |
| | $C_8$ | $59.3 \pm 2.0$ | $82.3 \pm 0.8$ |
| | $C_{12}$ | $74.8 \pm 0.9$ | $83.0 \pm 1.3$ |
| Sel. 4 | $C_4$ | — | $70.5 \pm 1.7$ |
| | $C_8$ | $50.1 \pm 3.2$ | $73.2 \pm 0.8$ |
| | $C_{12}$ | $58.3 \pm 1.6$ | $78.3 \pm 1.1$ |

A comparative study of meiotic behaviour and its relationship with seed fertility over the generations of autotetraploid *T. foenum-graecum* revealed that selection for improved seed fertility rather than vigour and vegetative traits of plants resulted in progressive improvement of meiotic stability. All the four genotypes followed the same pattern of meiotic stability leading to gradual diploidization, i.e. gradual decrease in multivalents and univalents with simultaneous increase in the frequency of bivalent formation.

Thus in autotetraploid *Trigonella* selection had a positive effect on the cytomorphological parameters. But improvement in seed fertility did not keep pace with the rate of meiotic stabilization and even after 12 years of selection seed fertility was still < 50% of their diploid progenitors. A survey of literature on autoploids revealed that cytomorphological studies were generally carried out only in a few early generations where there is rapid rate of meiotic stabilization and fertility improvement. Data on highly evolved and stabilized autoploids are, however, lacking. Our cytomorphological studies on advanced autotetraploid *T. foenum-graecum* indicated that though there is continuous improvement in cytomorphological traits, a plateau is reached for seed fertility beyond which there is no marked improvement. This is a major drawback for their commercial exploitation as a improved variety. Reports are available regarding genetical control on fertility [6, 7]. Many workers are of the opinion that poor fertility in autotetraploids can be mainly attributed to genetical–physiological disbalance of unexplained nature. It may be concluded that poor fertility in autotetraploids cannot be handled by the conventional selection method and some other genetical manipulations are necessary. Keeping this in mind, variability for fertility was created in the germplasm of autotetraploid *T.*

**Table 3. Growth and fertility parameters of different genotypes of 4x *Trigonella* in different generations in relation to their diploid progenitors**

| Parameter | Genotype | Character value (% of diploid) | | |
|---|---|---|---|---|
| | | $C_4$ | $C_8$ | $C_{12}$ |
| | Sel. 1 | 136.6 | 135.3 | 139.0 |
| | Sel. 2 | 167.8 | 115.9 | 127.8 |
| Plant height | Sel. 3 | 119.2 | 127.6 | 128.3 |
| | Sel. 4 | 117.4 | 118.5 | 123.7 |
| | Sel. 1 | 68.1 | 74.4 | 129.6 |
| | Sel. 2 | 82.7 | 93.3 | 135.5 |
| Number of branches | Sel. 3 | 58.3 | 60.4 | 113.2 |
| | Sel. 4 | 54.8 | 56.9 | 78.5 |
| | Sel. 1 | 65.8 | 67.8 | 79.2 |
| | Sel. 2 | 61.8 | 60.1 | 74.7 |
| Number of pods/plant | Sel. 3 | 72.5 | 75.4 | 83.2 |
| | Sel. 4 | 60.0 | 65.5 | 71.0 |
| | Sel. 1 | 32.7 | 32.9 | 33.2 |
| | Sel. 2 | 28.1 | 30.3 | 31.8 |
| Number of seeds/pod | Sel. 3 | 34.0 | 35.1 | 36.1 |
| | Sel. 4 | 27.5 | 27.4 | 28.9 |
| | Sel. 1 | — | 36.8 | 37.4 |
| | Sel. 2 | — | 21.7 | 22.6 |
| Seed yield/plant | Sel. 3 | — | 40.4 | 41.9 |
| | Sel. 4 | — | 20.1 | 21.3 |

*foenum-graecum* through hybridization and mutation. As a result, seed fertility has reached upto the diploid level in the vigorous lines of autotetraploid *Trigonella foenum-graecum* [8].

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