Mining *de novo* diversity in palaeopolyploids

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Palaeopolyploids per se are bestowed with buffering capacity to accommodate large-scale loss or gain of genetic material and they harbour *de novo* variations in the somatic tissues in higher order palaeopolyploids. Such *de novo* changes, when channelled into differentiation of daughter shoots become an important resource and valuable means to score diversity in the species, where sexual mechanisms are deficient. Using the palaeo-octaploid plant system *Mentha arvensis*, we demonstrate that somatic diversity accumulated *de novo* in asexually reproducing plants is elicited by polyploidization stress, unravelling the mine of hidden variation. This facilitates fixation of new genomic states in a short time, through their participation in bud-sport formation, that otherwise happens over the evolutionary timescale. The observations made have far-reaching practical implications, suggesting a simple means to realize a plethora of functionally balanced genetic variations at whole-plant level in evolutionary hybrids/natural polyploids/new polyploid lineages, without any involvement of the sexual process or other cumbersome means of induction of clonal variation that carries a risk of genotypic or aneuploid syndrome. The occurrence and impact of such processes in the evolution of natural hybrids is unknown, but it is believed that this may have contributed to the success and diversification of polyploid lineages.

Keywords: Asexual diversity, bud sports, *de novo* diversity, genetic enhancement, *Mentha arvensis*.

IN plant species where vegetative reproduction is obligatory, there exists an inherent mechanism to generate genetic variation *de novo* in their somatic tissues. Such *de novo* variation when channelled into differentiating shoots could give way to somatic mutations termed as 'bud sports'. Bud sports are somatic variants arising on a mother shoot during vegetative development. They could be valuable resource for genetic enhancement of species, where sexual means of reproduction are lacking/deficient for scoring variation. Bud sports are natural genomic filters and imminent means of genetic variation and speciation in asexually propagating plants[1–3]. However, the frequency of occurrence of such somatic variants is quite low in nature. One of the possibilities to enhance the frequency and spectrum of somatic variants could be through elicitation of inherent *de novo* variability, to realize stable variation. Such an elicitation is apparently possible by polyploid-mediated stress, because genomic stress may bring about genome shuffling between duplicating and segregating genomes[4,5], large-scale genomic changes caused by preferential genome/DNA sequence-specific elimination[6–9], and alteration in cytosine methylation[10], and therefore facilitate mining for newer genomic states. Further, when such genomic changes incur selective dosage amplification with respect to productivity/metabolic efficiency-linked genes, this would promise unique opportunities for improvement of vegetatively propagating plants, and may contribute to their evolutionary potential[11].

An ideal situation to this effect is observed in the palaeopolyploid species that reproduce obligatorily vegetatively. Such species are endowed with inherent capacity to accommodate large-scale gain or loss of genetic material and harbour individual *de novo* mutations that are sequentially accumulated during the asexual process. Their somatic tissues constitute a mosaic of chromosome complements enforced by somatic rearrangements. As such, the aberrant chromosome complements/somatic mutations that arise *de novo*, are the potential resource of new genomic states if developmentally passed through the somatic morphogenetic sieve and channelled into the formation of daughter shoots/bud mutations. Experiments conducted to explore such a possibility have met with startling revelations promising mining of *de novo* diversity, and the same are presented in this communication.

Material and methods

An elite diploid clone 'Kosi' of *Mentha arvensis* (family Lamiaceae, $2n = 8x = 96$) developed at CIMAP, Lucknow was used as the starting source material. Fast growing suckers with their axial buds physically exposed, were immersed for 7 h at 25°C in 0.1% aqueous solution of colchicine in 2% DMSO, followed by thorough washing in running water and planting in soil. Emerging plantlets were screened for leaf stomatal size and those with uniformly and distinctly enlarged stomata roughly twice the volume of source diploids, were isolated and multiplied for production of propagules (suckers) for planting. Initial examina-

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tion of the plants with enlarged stomata showed that these are autopolyploids ($4n = 16x = 192$), which gradually decay back to their original diploid chromosomal status ($2n = 8x = 96$) on maturity at ~100 days, but their stomata are still large (i.e. cytopolyploid status). Similar trend for ploidy decay is observed in suckers developed from autopolyploid shoots. Thus two types of sucker stocks are available for raising vegetative progenies and scoring of bud sports: (i) original source diploids ($2n = 96$), and (ii) polyploid-mediated cytopolyploid diploids ($2n = 96$, but with larger stomata). Both these sucker stocks were grown in nursery plots. Plantlets at 6–8 leaf stage were keenly examined to isolate morphological variants in vegetative progenies. Initial experiment on the scoring of bud sports was conducted in the cropping season of 2001, and repeated on larger scale during 2002–2004. Observations on essential oil yield of the stabilized bud sports were recorded in 2004 and 2005, on the plant population grown under optimal cultural conditions for this crop. Fresh herb obtained from the bud-sport variants and mother source at 120 days of growth was hydro-distilled in Clevenger's glass distillation unit for 2 h at 70°C to measure essential oil concentration. The dehydrated oil was analysed qualitatively by Gas Liquid Chromatography according to standard protocol on a HP 5890 series 2 gas chromatograph equipped with FID using fused silica capillary column.

Results

Observations recorded on a population of over 4000 plants each in the initial experiment revealed occurrence of only three variant morphotypes in the original diploid progeny at a frequency of 0.4%, whereas in the polyploid-mediated progeny there were 15 distinct variant morphotypes at the frequency of 2.0% in the first year. Thus there was clear enhancement not only in the frequency of occurrence of bud sports, but phenomenal enlargement in their spectrum by five fold. The experiment was repeated over three years, including two additional clones with reproducible results. As such, from the vegetative progenies of polyploid-mediated cytopolyploid progenies of clone

Figure 1. ***a***, *Mentha arvensis* plant showing bud-sport variation (left arrow, mother shoot; right arrow, variant shoot), ***b***. Variation in leaf shape of different bud-sport variants arranged in serial order from left to right. Top left corner is leaf from control, followed by variants nos 1–43. For the sake of uniformity in comparison, the leaf on the fifth node was taken into consideration and leaf shape differentiated on the basis of lamina symmetry, lamina form (apex, base and margin) according to the classification by Hickey[17]. ***c*** (all five figures in this row) Top view of some bud-sport variants depicting leaf morphology and topography in 3D view.

Table 1. Enumeration of qualitative variation for major secondary metabolite (essential oil) in bud-sports of *Mentha arvensis*

Genotype	Essential oil concentration in the fresh herbage (%)	Major constituents in the essential oil (%)			
		Menthone	Iso Menthone	L-Menthol	Menthyl acetate
M. arvensis clone 'Kosi'	0.83	1.40	2.72	76.75	8.43
Variant 1	0.75	7.68	2.46	69.17	6.88
Variant 2	0.75	8.04	2.37	75.26	4.72
Variant 3	0.98	14.40	2.80	56.04	9.34
Variant 4	1.12	4.05	2.70	71.96	10.27
Variant 5	0.65	2.30	4.04	77.83	0.25
Variant 6	0.60	2.11	4.22	78.38	2.41
Variant 7	0.50	1.62	3.82	79.99	2.53
Variant 8	0.75	2.71	3.12	68.28	15.16
Variant 9	0.80	4.69	3.83	76.20	4.82
Variant 10	0.87	4.36	2.72	76.07	6.80
Variant 11	0.77	1.55	2.49	82.50	4.79
Variant 12	0.62	1.39	3.45	72.98	11.93
Variant 13	0.87	2.99	2.64	74.32	9.98
Variant 14	0.65	4.32	2.95	71.41	6.73
Variant 15	1.02	2.91	3.08	78.41	4.63
Variant 16	0.80	2.58	4.26	76.92	4.20
Variant 17	0.65	1.73	4.94	61.27	19.36
Variant 18	1.00	4.32	2.95	71.41	6.73
Variant 19	1.25	4.59	2.92	74.86	6.95
Variant 20	0.57	4.49	2.78	58.55	20.00
Variant 21	0.80	2.10	4.02	70.40	10.11
Variant 22	0.65	2.72	4.19	76.11	5.39
Variant 23	0.60	3.51	3.04	72.23	9.85
Variant 24	0.92	4.70	3.89	72.27	6.88
Variant 25	1.00	7.07	2.86	74.10	4.40
Variant 26	0.80	8.79	2.89	73.61	3.30
Variant 27	0.87	6.23	3.70	69.44	9.40
Variant 28	0.77	4.44	4.27	60.73	19.21
Variant 29	0.97	2.90	3.23	75.75	7.75
Variant 30	0.60	3.03	3.96	68.20	6.76
Variant 31	1.05	19.62	3.48	48.75	14.88
Variant 32	0.75	5.68	3.41	69.48	7.44
Variant 33	0.84	3.15	4.55	72.54	8.31
Variant 34	0.80	2.81	3.08	80.12	4.76
Variant 35	0.78	2.57	3.06	79.31	4.88
Variant 36	0.82	3.48	3.20	70.57	11.76
Variant 37	0.78	11.89	3.01	67.28	5.53
Variant 38	0.70	2.90	2.50	78.73	6.71
Variant 39	0.80	22.33	3.89	47.87	11.80
Variant 40	1.00	4.72	2.95	68.64	12.56
Variant 41	0.80	6.43	2.89	68.67	12.47
Variant 42	0.75	14.73	3.18	55.56	14.20
Variant 43	0.75	6.20	4.32	69.11	7.40
C.V.	19.47	109.85	19.5	11.17	63.56

'Kosi', 43 types of distinct bud-sport variants differing in overall plant exomorphology and variation in leaf shape (lamina symmetry, apex, base and margin), and quantitative and qualitative variation in essential oil profile were realized in the population of 20,000 plants scored. A representative plant depicting bud-sport derived variant shoot *vis-à-vis* mother shoot, and variation in leaf lamina morphology are shown in Figure 1, and qualitative variation realized in secondary metabolite (essential oil) are enumerated in Table 1. Observations suggest that genomic stress enforced by polyploid-mediated pressure may be eliciting *de novo* genomic/somatic instability and commensurately higher recovery and fixation of newer genotypic state per se at organism level.

Discussion

Most angiosperms are considered to have palaeopolyploid genomes resulting from one to several rounds of genome doubling during speciation and diversification[12–14]. Considerable attention has been paid to understanding genetic events associated with early allopolyploid nucleus formation, indicating that genetic and epigenetic changes frequently

linked with polyploidy potentially influence the balance of gene expression and metabolism[9].

The presence of multiple genomes bestows buffering capacity to the palaeopolyploids/evolutionary polyploids to withstand large-scale gain/loss or alteration in their genetic material. Therefore, any inter-genomic interaction and genomic shuffling enforced in higher order palaeopolyploids could be a valuable resource for genetic diversification and fixation of new genomic states. In fact, there are in-built features in such species that generate somatic variation *de novo*, which when channelled into formation of vegetative shoots bring about bud-sport mutations to realize fixable genetic variability[2], although the incidence of such bud sports is quite low in nature. Therefore, search for factors that could enhance the occurrence of bud-sport variations could add value to genetic improvement strategies for vegetatively propagating plants. Since such variations that are apparently parallel to evolutionary variation are common in callus cultures over the passage of time, and their frequency is distinctly higher in polyploids[4], accordingly, polyploid-mediated genomic stress to realize enhanced somatic variation in the vegetative tissues was considered as a prospective possibility in the present study.

In *M. arvensis* itself, search for bud sports has been one of the principal means to facilitate variation and genetic improvement[15]. The present study aimed at eliciting *de novo* variability by applying polyploid-mediated genomic stress has opened newer prospects to mine hidden somatic variation to isolate genetically fixable variants in palaeopolyploids/higher order evolutionary polyploids, where sexual means to achieve variations are lacking or deficient. It has earlier been reported with respect to chromosomal variation in callus cultures, that even for aneusomatic calluses, it is mainly the euploid/genetically balanced cell that is able to pass through the morphogenetic sieve to enter into organogenesis[16]. Presumably, a similar phenomenon may be operative during bud-sport formation, wherefore the frequency and spectrum of bud-sports are distinctly enhanced under polyploid-mediated stress on account of enhanced *de novo* chromosome variation[16] and/or genomic shuffling/somatic recombination[5], preferential genome/sequence-specific elimination[9]. However, only competent cells with balanced new genomic state are able to differentiate into variant daughter shoots[16]. Such developmentally competent variant shoots/bud sports are stable and suitable for commercial utilization.

The observations recorded here clearly suggest that the genetic diversity accumulated in the somatic tissues *de novo* could be elicited by polyploid-mediated genomic stress to unravel a mine of hidden variation in plants lacking sexual recombination, providing immense opportunities for genetic improvement of asexually reproducing species. The study has practical implications as it suggests a simple means to realize vast variation at the whole-plant level, imitating evolutionary change without any involvement of the sexual process or other cumbersome means of induction of clonal variation. Further, because the bud-sport variations are an outcome of *in vivo* somatic selection, their vegetative progenies are expected to be biologically competent, unlike the aberrant regenerants originating from *in vitro*-driven variations that generally suffer from aneuploid syndrome.

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