Synthetic hybrid speciation: a resource for breeding novel lineages for secondary metabolites

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Abstract

Whereas the splitting of ancestral lineage into daughter species remains a common course of speciation, but hybridizing the two related species to give rise to a third independent lineage could be a powerful tool to hybrid speciation. The latter could give way to new plant type/s featuring novel combination for physiological, developmental, and metabolic pathways. Pre-selection of progenitor parental genotypes could enable realization of hybrid lineages with predictable features to a large extent. At the same time such derived lineages shall ensure hybrid fixity through vegetative propagation, but could offer opportunity of seed cultivation through ploidy mediation for fixation and/ or variation through meiotic segregation for selection of novel types. Experimental manoeuvring imitating homoploid speciation or somatic variation could be the other means to release de novo variation. In view of above, a prospective outline is discussed towards qualitative breeding for secondary metabolites.

Keywords Synthetic hybrid speciation · Somatic variation · Breeding interspecific hybrids · Allopolyploid lineages · Qualitative breeding for secondary metabolites

Introduction

One of the key realizations in the genomics era is that evolution and speciation in flowering plants has been set in motion by multiple repeated episodes of whole-genome doubling or polyploidization, referred to as 'wondrous cycles of polyploidy in plants' [31]. Notwithstanding, the occurrence of such multiple cyclical incidences, the ancient polyploidization have not exhibited presence of high chromosome number or enlarged genome size. Instead, the supposed accretion of chromosomal material realized through such ancient events of polyploidy, is partially reversed over time through massive chromosomal rearrangements, reductions in chromosome number and large-scale loss of both repetitive and duplicate genes, all leading to downsizing of nuclear

material [3, 13] through repeated “wash–rinse–repeat” cycle of polyploidization followed by non-random and incomplete diploidization [31]. While the term 'polyploidy' was coined by Winkler [33], immediately thereafter Winge [32] put forwarded a theory that hybridization followed by chromosome doubling (i.e., amphidiploidy) would enable survival of interspecific hybrids through availability of homologous chromosome partners. A lot has since been discussed about the occurrence and consequences of polyploidy as a source for evolutionary innovation and diversification [28] in speciation, and generation of new plant types. The latter have been utilized as a resource for creating novelty, and taping of certain characters from wild relatives to some extent. Further, it is surmised that both auto- and allo- polyploidy hold great promise in value addition to plants, especially where the plant biomass is the source of economic product, and their active metabolite components are valued in industrial applications. This article intends to underscore the prospects of synthetic hybrid mediated genetic and genomic interventions for breeding novel lineages for secondary metabolites.

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Secondary metabolites in perspective of natural resources for value addition

In order to withstand environmental pressures, plants produce an arsenal of structurally diverse chemicals, often called as secondary metabolites [17]. Such metabolites are synthesized near the stationary phase of growth and are secreted in specific tissues / vacuoles, and / or translocated to the storage tissues. They are not directly involved in the normal growth, reproduction, and development, but they do induce flowering, fruit set and abscission, and maintain perennial growth habit. More importantly, they generally mediate ecological interactions conferring selective advantage for the organism by increasing its survivability, performing the role of attractants or, conversely, as repellents that make them competitive in their own environment.

Over 50,000 secondary metabolites have been discovered in the plant kingdom. Such natural products continue to remain main sources of drugs, food additives, and powerful indicators of the biosynthetic capacity of plant metabolism.

In the past two decades, the engineered biosynthesis of many plant natural products have been achieved through the construction of microbial cell factories [35]. Alternatively, it has been shown that plant pathways can be modified in the native producer with a variety of metabolic engineering approaches [23]. However, a more natural means could be to harness the target metabolites through tapping the metabolite diversity per se or reproductive intervention thereof for breeding new lineages.

What is unique to breed for secondary metabolites

Production of plant-based modern drugs, health care, immuno-modulation and beauty products has become an important segment of the pharmaceutical and aroma industry. Notable single active molecules as well as blended combinations are in use in such formulations. This necessitates to make directed scientific efforts to breed for plant type/s that are rich for a given specialized active phyto-molecule to facilitate isolation of the desired molecule from the crude concentrate.

The raw material and the active ingredient concentrate are obtained from the whole plant, mainly from vegetative biomass and / or storage tissues. Therefore, to realize high productivity of the economic product i.e., target active molecule/s, the primary goal of the plant breeder remains to realize enhanced concentration of the secondary metabolite of choice in the plant biomass. Another important

feature worth considering while breeding for secondary metabolites is that the resource plants owe the principal mode of their propagation through vegetative propagules / or could be efficiently propagated asexually for commercial cultivation. As such, any improved genetic stock once realized does not pose problem of genetic instability, although could be a resource for harnessing variation through seed progenies. Besides, such obligate asexual plants are loaded with inherent de novo genetic diversity [18], that could serve as a mine to tap novel variations.

Interspecific hybrids are the valuable resource

Hybridization between related species has been the common speciation event giving rise to reticulate phylogenies mediated through autopolyploidy, allopolyploidy or homopolyploidy. Whereas hybridization between land races of a biological species is quite frequent, but there are instances where interspecies hybridization could yield partially fertile F_1 hybrids. The latter could become a valuable resource in generating new combinations. Further, if the hybrids could be developed between parents of choice, then a valuable plant type can be developed. Moreover, such hybrids could serve as a treasure to exploit newer combinations and isolate desirable features that enhance value to the biological material. The following scheme of thing may be worth exploring:

(a) F_1 Interspecific hybrids:

In deliberating on the potential of interspecific hybrids for secondary metabolite prospecting / biogenic pathway complementation, it may be pertinent to cite the instance of the model systems of mints—the *Mentha* spp. valued across all the five continents for their aromatic essential oil and its qualitative components. The literature on this genus is replete with examples on the occurrence of natural as well as artificial interspecific hybrids, and transgressive variants / complement fractionation for essential oil constituents [24, 26]. Tucker and Kitto [26] provide an exhaustive list relating to genetic inheritance of monoterpenes in *Mentha* based on analysis of the paired sets of strains that allow the conversion to occur on one hand and prevention to conversion on the other in the biosynthetic pathway. While discussing the wild relatives for cultivar improvement in mints, Tucker and Kitto [26], Vining et al. [30] pinpoint that there are 18 species, 31 subspecies or botanical varieties. Five species in the section *Mentha*, namely *M. aquatica*, *M. arvensis*, *M. longifolia*, *M. spicata*, *M. suaveolens* have produced 11 recognized hybrid species, including Peppermint (*Mentha* × *piperita*), Scotch spearmint (*M.* × *gracilis*), Spearmint (*M.* × *spicata*), Japanese corn mint (*M. canadensis*, often called as *M. arvensis* L. forma *piperascens* Holmes—an important source of natural menthol) and American spearmint (*M.* ×

villosa-nervata), are cultivated in various parts of the world for their essential oil valued in pharmaceutical and aromatherapy applications and culinary properties [26, 27, 30]. The chromosome number reported in this genus varies from $2n=24$ to $2n=96$ suggesting the base number of $x=12$, with some variations in interspecific hybrids reaching up to $2n=120$ [5]. The representative examples cited from above are: $2n=24$ (*M. longifolia*) to $2n=48$ (*M. spicata*), $2n=72$ (*M. x piperita*, *M. arvensis*), $2n=96$ (*M. arvensis*, *M. aquatica*); although some stray instances of other divergent numbers are also encountered in natural populations and progenies of interspecific hybrids. Since all such species exhibit variation in qualitative profile of essential oil and reproductive features, therefore efforts have been made to combine qualitative features of important species through interspecific hybridization. Murray et al. [15] provide a categorical account on the generation of range of qualitative variations consequent upon interspecific hybridization between *M. aquatica* x *M. spicata* likened to *M. x piperita*. Tucker and Fairbrothers [25] provide a unique instance of artificial hybrid synthesized from a cross between *M. arvensis* ($2n=72$) x *M. spicata* ($2n=48$), otherwise likened to *M. x gracilis* postulated to be a hybrid clonal complex, whereby the F_1 progenies exhibited incidence of somatic chromosome numbers of $2n=48, 60, 72, 84$, and 96 that are commensurate with euploid series. Although most of such hybrids are sub fertile or sterile but vigorous means of vegetative propagation enables them to persist as well as facilitate production of new forms [5], bud sport variants [10]. In India, efforts have been made to develop interspecific hybrids between *M. arvensis* and *M. spicata* [8, 20], *M. arvensis* and *M. x piperita* to combine the desirable features of the two parents [9]. It is worth pointing out that even in the absence of sexual reproduction, the desirable hybrids could perennate through vegetative propagation to overcome the difficulties in commercial plantations, but at the same time could be valuable resource for further improvement through sexual intervention. This is discussed below for further exploration.

(b) Pre-selection of putative parental genotypes for harnessing the interspecific hybrid lineages:

Modulation of secondary metabolic pathway could be engineered to some extent through tools of system biology, but it should be preferable if could be done through natural means of genetic manipulation from a cultivation perspective. Interspecific hybridization between the species sporting common biosynthetic pathway for secondary metabolites may bring out complementation to realize enhanced production of desired metabolite / new component(s). It would be more rewarding if it should be possible to select for progenitor donor genotypes that are enriched with complementary secondary metabolite component that are likely to complement desirable features in the interspecific hybrid.

The combination of both qualitative and quantitative characters has earlier been realized through interspecific hybrids of aromatic grasses (*Cymbopogon* group), leading to development of the hybrid clones, namely CKP-25- i.e., *C. khasianus* x *C. pendulus*, and Jamarosa i.e., *C. jwaruncusha* x *C. confertiflorus* [7]. More recently, Srivastava et al. [21] has reported the occurrence of huge variation in qualitative profile in the essential oil of *Ocimum basilicum* differentially enriched either for methyl chavicol, methyl eugenol, linalool, methyl cinnamate, promising the development of diverse varieties for commercial cultivation. Further, to fix this natural variability and to enhance the growing potential of such variant genotypes from annual to perennial, interspecific hybrids are developed between *O. basilicum* after inter-crossing this with cold tolerant *O. kilimandscharicum* to change it to evergreen genotype [2].

(c) Amphidiploids as valuable resource for breeding new lineages:

The interspecific hybrids could bring compatible metabolic pathway combinations between the pre-selected donor genotypes, but to ensure their reproductive efficiency through seed cultivation, it is desirable to develop amphidiploids in such interspecific hybrids that remain sterile at F_1 level. Such amphidiploids would not only bring seed fertility, but also promise another round of opportunity to score for novel lineages for secondary metabolite profile in the segregating populations. The present authors (unpublished data) have successfully developed seed fertile amphidiploids between *O. basilicum* x *O. kilimandscharicum*, supporting evergreen growing habit and fixation of metabolite profile character (Fig. 1a, b), and are currently involved in scoring F_2 progenies for further variation and fixation to isolate better types. Besides induced amphidiploids, it would be worth examining to score for natural seed formation in the F_1 hybrids that may happen owing to unreduced gamete formation and reorganization of karyotype [19], even though at a very low level.

(d) Incidence of somatic and homoploid variation

In plants, where asexual mode of reproduction is obligatory, they resort to alternative mode of speciation through participation of de novo variation *in planta* in the formation of variant shoots [18]. Such variation is more likely to happen in the sterile F_1 interspecific hybrids / nascent allopolyploids [34]. The findings of Tucker and Fairbrothers [25] are clear case of such example. Going beyond the taxonomic boundaries, a different instance to such effect has been observed in the sterile hybrid between *Cymbopogon jwaruncusha* x *C. confertiflorus* by us. In a particular hot and humid season in the month of September under Lucknow conditions, profuse emergence of variable vegetative propagules emanating from inflorescence stalk was observed in this hybrid (Fig. 1 c).

Fig. 1 a–b. The evergreen inter-specific hybrid between *Ocimum bacilicum* x *O. kilimandscharicum* combining qualitative features of essential oil of the former and perennial habit of the latter, a. F₁ sterile hybrid, b. fertile amphidiploid; c. the F₁ interspecific hybrid

between *Cymbopogon jwaruncusha* x *C. confertiflorus* showing variable vegetative propagules (arrow marked) emerging from the inflorescence stock

Although homoploid formation is quite rare, but could be occasionally observed in 'nature' through hybridization of chromosomally divergent parental species and subsequent fixation of a novel combination of chromosome fusions/fissions in hybrid descendants or could be through radiations [14]. If it happens, can produce new and unusual combinations and lineages. It will be worthwhile to cite an example of *Mentha* x *piperita*, where—seed formation is observed in a few clones of this otherwise sterile hybrid [9], possibly mediated through homoploid system of balanced gametogenesis.

(e) De novo somatic variation and somatic reduction:

If polyploidization constitutes an important process in the evolution, it is also possible that the same could be reversed. Alongside with increasing of DNA content, chromosome

numbers and of entire genomes in somatic tissues, there are numerous facts of opposite processes—loss of certain chromosomes, alleles, or whole genomes by somatic cells [22]. Chromosome elimination in somatic tissues of distant hybrids is a well-documented process [4, 16, 29].

It is quite likely that an interspecific hybrid planned to complement secondary metabolite pathway could be further channelized to reduce the genetic load but at the same time generate new lineages and new combinations for harnessing the qualitative and quantitative profile of the active principles of choice. Incidence of somatic chromosome number $2n=48$ in the F₁ progenies of interspecific hybrid between *M. arvensis* ($2n=72$) x *M. spicata* ($2n=48$) otherwise supposed to contain $2n=60$ points to the possibility of process of somatic reduction or chromosome elimination.

Sharma [18] propounded a new concept of speciation for asexually reproducing plants suggesting that somatic tissues in such plants represent a mosaic of somatically variant cells owing to mitotic errors; and any participation of variant cells in formation of daughter shoots could give rise to new variation and speciation. Lavania and Srivastava [11, 12] while studying the patterns of variation in callus cultures over a passage of time showed that owing to mitotic errors there are both aneuploid and euploid variation that are apparently parallel to evolutionary process in 'nature', but at the same time only balanced euploid cell evince the potential of differentiation. It is likely that similar variation may happen *in planta* during vegetative propagation of F_1 interspecific hybrids. Any variant cell that enters into differentiating meristem, may give rise to new variant shoot / bud sport and a new plant type and newer combination for secondary metabolite pathway. It is important to note that the tendency of generating chromosomal variation could be enhanced if the cell / callus cultures are treated with chemicals that may interfere with spindle apparatus. The chemicals like *para*-fluorophenylalanine [6], caffeine [1] have been found to induce somatic reduction promising new interspecific genotypes when applied to callus cultures / or even applied to proliferating tissues in the vegetative propagules.

It may be emphasized that interspecific hybridization could bring combination and / or complementation of the donor characters / breeding habit / secondary metabolic pathway, but the value of such hybrids could be enhanced if the donor parents are preselected for qualitative characteristics for secondary metabolite that may be attendant in the diverse populations. Thus, such hybrids could serve as a valuable resource to release new lineages mediated either through incidences of partial fertility, or natural / artificial manoeuvring of somatic rearrangements / complementation, or variation released from the segregating progenies consequent to amphidiploidization.

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Declaration

Conflict of interest Authors declare no conflict of interest.

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