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IMPROVED TECHNIQUE FOR CHROMOSOME STUDY IN SOME MEMBERS OF LABIATAE

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IMPROVED techniques were developed for the study of chromosome in some genera, such as *Ballota, Coleus, Lavandula, Mentha, Ocimum, Salvia* and *Scutellaria* of the family Labiatae. Three different mixtures containing α-bromonaphthalene, aesculine and saponin, p-dichlorobenzene, aesculine and 8-hydroxy-quinolene and a third one containing only aesculine were found to be the most suitable combinations of pretreating chemicals in these genera.

The members of the family Labiatae, being aromatic, contain several types of essential oils in their cytoplasm. As a result, the difficulty in the study of their chromosomes, usually very small in size, has been strongly felt since long. The oil contents of the cytoplasm are considered to prevent the penetration of the pretreating chemicals to the cytoplasm and thereby keeping the viscosity balance between the cytoplasm and spindle mechanism and the metaphase chromosome system unaffected[1]. The various investigations, so far carried out in these species, have not been able to give the details of their chromosome structure. With this view in mind, different pretreating agents alone and in combinations were tried on 8 genera consisting of 21 species and 40 varieties and populations of Labiatae, collected from different parts of the world.

For the study of somatic chromosomes, several pretreating chemicals and their combinations were tried for different species and genera. Of these, a freshly prepared 1 : 1 mixture of α-bromonaphthalene and aesculine with a slight addition of saponin was found to be the most successful in the species of *Ballota, Coleus, Lavandula, Mentha, Ocimum* and *Salvia*. A drop of α-bromonaphthalene was taken in water and shaken well to prepare its homogeneous mixture. To this a bit of saponin was added and again shaken to complete the saponification. Mixture of aesculine was separately prepared by adding to water a little bit of aesculine powder with the help of a needle to produce just a blue tinge. These two mixtures were then mixed in a 1 : 1 ratio. Pretreatment was then done with fresh and healthy root tips kept in this mixture and chilled for 3 min at 4°C. After cold treatment, the pretreating mixture with root-tips were kept at 14–16°C for 1 to 2 hr depending upon the number of chromosome in the different species[2]. However, to get the desired results in *O. gratissimum* in the saturated aqueous solution of p-dichlorobenzene, a trace of aesculine was added in the manner described before and then 2 drops of 8-hydroxyquinolene was mixed. The mixture of these pretreating agents with root-tips were kept at 4°C for 5 min and then transferred to 16°C for 2 hr . In the various species of *Scutellaria*, satisfactory results were obtained in an aqueous mixture of aesculine only[5] (table 1.) All the mixtures were prepared fresh and in tap water.

During the course of this study, certain interesting observations were made. It was found that the aqueous mixture of α-bromonaphthalene, aesculine and saponin was successful in 32 varieties and populations under 15 species and 7 genera. All the species and populations of *Ocimum* responded to this mixture alike, except the 2 populations of *O. gratissimum* (table). In all these cases, good metaphase plates with fine clarity of chromosomes were obtained. Despite their small size, primary and secondary constructions and satellites were clearly observed in the chromosomes and they were counted with great ease due to their fine scattering in the semisolid cytoplasmic background.

In the species with high essential oil contents (*Ocimum, Mentha*), saponin comparatively acted well. It can be suggested that saponin clears the cytoplasm, thereby facilitating the penetration of the pretreating chemicals.

Almost all the species under investigation showed that they needed the duration of pretreatment according to their chromatin contents or chromosome num-

Table 1 *Data obtained in pretreating chemicals and combinations for various species and genera.*

Species	Pretreating chemicals	Chilling		Duration hr	Temperature °C	Chromosome number observed 2n
		at °C	for min.			
Ballota nigra	α-Bromonaphthalene, aesculine and saponin	–	–	1½	14–16°C	18
Coleus blumei	-do-	–	–	1½	"	30
Lavandula latifolia	-do-	–	–	2	"	48
Mentha aquatica	-do-	–	–	1½	"	96
M. pulegium	-do-	–	–	1½	"	20
Ocimum americanum						
Populations I–IV	-do-	4	3	2	"	72
Population V	-do-	4	3	2	"	84
O. basilicum	-do-	4	3	2	"	48
O. basilicum var. *glabrum*	-do-	4	3	2	"	52
O. basilicum var. *pilosum*	-do-	4	3	2	"	48
O. canum						
Populations I–III	-do-	4	3	1½	"	24
Populations IV–V	-do-	4	3	1½	"	26
O. carnosum	-do-	–	–	1½	"	48
O. gratissimum Populations I & II	p-dichlorobenzene, aesculine and 8-hydroxyquinoline	4	5	2	16°C	40
Population III	-do-	4	5	2	"	88
Ocimum kilimandscharicum Populations I & II	α-Bromonaphthalene, aesculine and saponin	4	5	2	16°C	76
O. sanctum						
Populations I–IV	-do-	4	3	1½	14–16°C	32
Populations V–VI	-do-	4	3	1½	"	34
Populations VII	-do-	4	3	1½	"	36
O. viride	-do-	–	–	1½	"	38
Salvia aethiopis	-do-	–	–	1½	"	22
S. glutinosa	-do-	–	–	1½	"	16 or 18
Scutellaria adenostegia	Aesculine	5	5–10	3½	10–12°C	22
S. alpina	-do	5	"	"	"	28
S. altissima	-do-	5	"	"	"	30
S. haematochlora	-do-	5	"	"	"	20
S. intermedia	-do-	5	"	"	"	20
Teucrium montanum	-do-	5	"	"	"	22

bers. Species with low chromosome number required comparatively shorter duration, while spp. containing high chromosome numbers needed longer durations.

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