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PAPER ON ANTHER AND MICROSPORE CULTURE OF SRI TOBACCO

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Abstract

Anther culture plays an important role in getting haploid plantlets via either organogenesis or embryogenesis. In addition, microspore culture has also great contribution for producing haploid plants through directly culture on the medium. In the present study, the experiment was evaluated for recording Which Nitsch medium (liquid or solid) is the best for the anthers of *Nicotiana tabacum* for producing haploids plants, the effects of the anther walls in *Nicotiana tabacum* formation of haploids plant in liquid medium and evaluating the effects of different stages (satge1-4) *Nicotiana tabacum* buds for haploid production. In this experiment two different types of medium (solid and liquid) were used. The solid medium was conducted in quadruplo, whereas the liquid medium was conducted in triplo. The four different sizes (stage1: <17mm, stage2: between 17-20mm, stage3: between 20-23mm and stage: 4 >23) *Nicotiana tabacum* buds were used. The results were calculated on the basis of percentage. Among three media, the highest planlets were observed in solid medium. In the solid medium, the highest plantlets were found in stage 4 (all petri-dishes above 50% except1), while the lowest plantlets were observed in stage1 (all petri-dishes zero, except1). In the stage2, above 50% planlets were found in each petri-dish except 1, while below 50% planlets were observed in all petri-dish except 1 in stage3. The highest plantlets were examined in stage2 (above 50% except1), whereas the lowest plantlets were stage3 (Only one petri-dish contained plantlets) in liquid medium which containing A+M. In the liquid medium (M) only one petri-dish contained planlets, whereas other two petri-dishes were empty in stage1.The other three stages were empty. Our results indicated that the best Nitch media were solid medium than others for producing haploids plants. Among the four different stages, stage2 and stage3 was the best both in solid and liquid medium (A+M).The effects of the anther walls has great impact for producing haploid plantlets in all stages except stage3.

Keywords: Tobacco (*Nicotiana tabacum*), Anthers, Microspore, Liquid and Solid medium.

Introduction:

Anther culture is a strategy in which different stages of anthers aseptically taken from unopened flower bud and are cultured in vitro nutrient medium resulting in getting haploid plantlets via either organogenesis or embryogenesis. In microspore culture is also a technique for producing haploid plants through directly culture on the medium. In Plant Breeding, anther culture is an important method for producing haploid. Haploid plants contain only one set of chromosome that helping to identify recessive mutation. Homozygous plant can be easily get one generation through haploid plant production by double haploid via chromosome doubling. On the other hand, in conventional breeding methods require more time up to six generation to produce homozygous plant.

Haploid plants could be originated either through androgenesis or gynogenesis. In gynogenesis process, haploid plants are derived from ovules or ovaries that are not fertilized. Similarly, haploid plants are originated either through anther or microspore culture directly or indirectly. Androgenesis process was used in this experiment.

For some species (*Nicotiana tabacum*) micospores could be convinced to variation from the gametophytic pathway (helping to normal pollen development) to sporophytic pathway (aiding to embyo or callus formation). In this process, haploid plants are obtained either through anther culture or microspore culture.

The goals of this experiment were:

1) Which Nitsch medium (liquid or solid) is the best for the anthers of *Nicotiana tabacum* for producing haploids plants.
2) The effects of the anther walls in *Nicotiana tabacum* formation of haploids plant in liquid medium.
3) Evaluating the effects of different stages (satge1-4) *Nicotiana tabacum* buds for haploid production.

Materials and Methods:

Sterilization of flower buds was done in the first day. Flower buds of various lengths were transferred into a sterile beaker. 70 % ethanol was added to the beaker and after a few seconds pour off the ethanol (to remove air and fatty layers from the bud surface). Moreover, 2% sodium hypochlorite (NaOCl) solution was added (1/1 diluted commercial bleach) and incubate for 10

min while swirling the solution in the beaker several times. Pour off the hypochlorite and added sterile water. Swirl and pour off the water was done. Sterile water was added and repeated this washing step, all together 3 times.

In case of flower buds staging, the buds were left in sterile water after the last washing step. The length of each bud was measured (from the flower base to the tip of the petals), using graph paper which is put underneath a petri-dish. The buds were distributed (12 buds per stage are needed) over four (9-cm) petri-dishes, containing a few ml of sterile water to prevent drying, according to the following stages: stage 1: < 17 mm, stage 2: between 17 –20 mm, stage 3: between 20-23 mm and stage 4: > 23. Anthers preparation were done in the following procedures. A flower bud was transferred to a sterile (6 cm) petri-dish with some sterile water (to prevent drying). The bud was opened with a scalpel and removed the anthers (each bud has 5 anthers). The anthers were not damage. The anther filaments were not taken. Two anthers were transferred from each bud to solid medium and the remaining three anthers to a 6 cm dish with 5 ml liquid medium. Dishes with solid medium should contain 6 anthers (from 3 buds of the same stage) and dishes with liquid medium 12 anthers (from 4 buds, so two anthers of the last bud are not used). The dishes were sealed with parafilm and incubated the cultures at 28 °C in darkness. After 15 or 16 days later, in many cases, anthers in liquid medium have released microspores. By removing the anthers one removes both inhibiting and promoting effects of the anther walls. Since not all microspores have been released the further culture of the anthers allows the evaluation of the inhibiting and promoting effects. The anthers were transferred from liquid medium to new petri-dishes with fresh liquid Nitsch medium. The dishes were incubated from which the anthers have been removed (containing released microspores) at 25°C in the light, together with the solid media plates and the anthers in fresh Nitsch medium. After three weeks later, determination: (i) on the solid medium plates: the number of anthers that formed plantlets and (ii) in the liquid media: the number of embryos and plantlets that have been formed. Evaluate the effect of stage and culture procedure on the yield of haploid plants.

Results:

In this experiment two different types of medium (solid and liquid) were used. The solid medium was conducted in quadruplo, whereas the liquid medium was conducted in triplo. A+M represented that both the anthers and microspores present, permitting the anthers to utilize their inhibitory as well as stimulating effects on the microspores. On the other hands, M indicated that

only microspores were present, permitting the microspores that were free from the anther effects for freely growing in the medium. The four different sizes (stage1: <17mm, stage2: between 17-20mm, stage3: between 20-23mm and stage: 4 >23) *Nicotiana tabacum* buds were used. The results were calculated on the basis of percentage. The formation of plantlets that were observed in various media could also be found in Figure1, Figure2 and Figure3.

Table1: Haploid plantlets formation of different stage and culture Nitsch medium in *Nicotiana tabacum*

	SOLID MEDIUM(A+M) %				LIQUID MEDIUM (A+M) %			LIQUIDMEDIUM(M)		
STAGE1	16	0	0	0	8	50	100	33	0	0
STAGE2	83	33	66	50	3	100	66	0	0	0
STAGE3	33	33	16	66	33	0	0	0	0	0
STAGE4	66	100	33	50	5	5	33	0	0	0

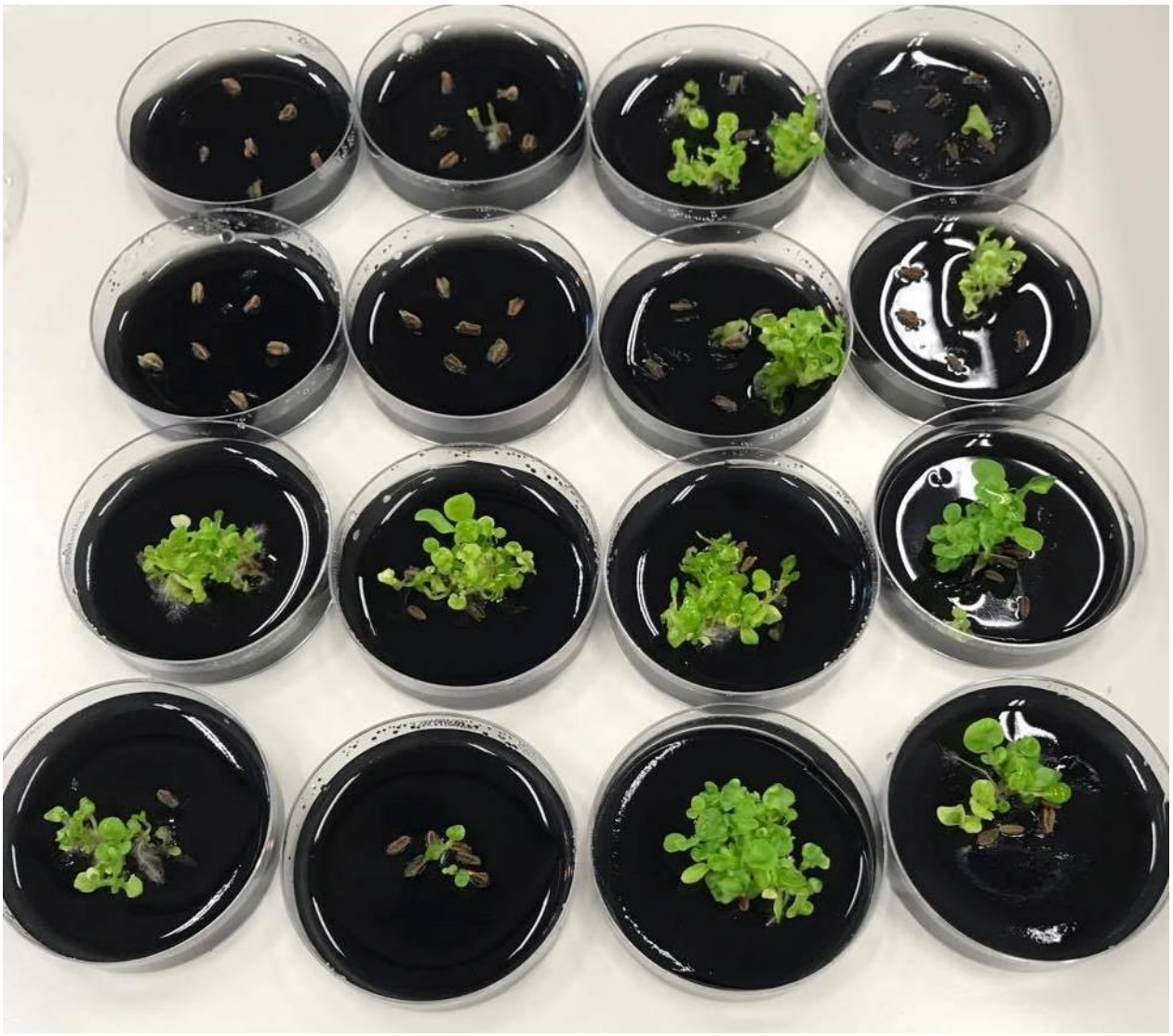

Figure1: Haploid plantlets were produced in solid medium in different stages (STAGE1-4).

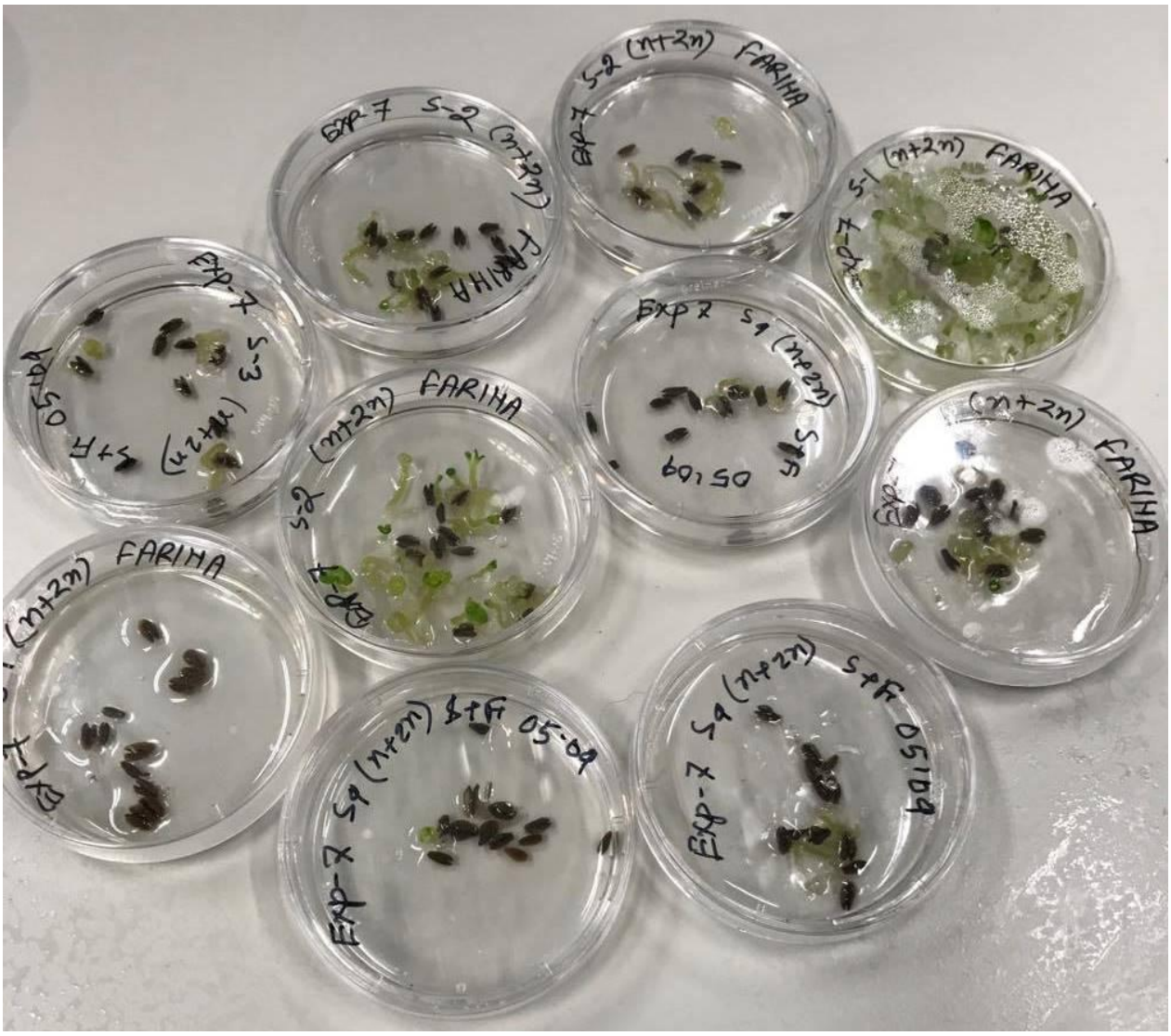

Figure2: Haploid plantlets were produced in liquid medium (A+M) in different stages (STAGE1-4).

Figure3:Haploid plantlets were produced both in solid and liquid (A+M) medium in different stages (STAGE1-4).

Discussion:

Among three media, the highest planlets were observed in solid medium. In the solid medium, the highest plantlets were found in stage 4 (all petri-dishes above 50% except1), while the lowest plantlets were observed in stage1 (all petri-dishes zero, except1). In the stage2, above 50% planlets were found in each petri-dish except 1, while below 50% planlets were observed in all petri-dish except 1 in stage3. The highest plantlets were examined in stage2 (above 50% except1), whereas the lowest plantlets were stage3 (Only one petri-dish contained plantlets) in liquid medium which containing A+M. In the liquid medium (M) only one petri-dish contained planlets, whereas other two petri-dishes were empty in stage1.The other three stages were empty.

Conclusion:

From the above discussion, it was cleared that the best Nitch media was solid medium than others for producing haploids plants. Among the four different stages, stage2 and stage3 was the best both in solid and liquid medium (A+M).The effects of the anther walls has great impact for producing haploid plantlets in all stages except stage3.

Figure5: Haploid plantlets were produced both in solid and liquid (A+M) medium in different stages (STAGE1-4).