Evaluation of genetic variability in *Spathoglottis* species: A model orchid

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**ABSTRACT**

*Spathoglottis* species are popular terrestrial orchids used as model for orchid studies. Due to alarming threat to species diversity, loss of habitat and increasing popularity, it needs to establish information on morphological characteristics as well as genetic variability for qualitative and quantitative traits to utilize for the genetic improvement programme and conservation of these endangered species. With these objectives an attempt was made to estimate genetic variability in terms of genetic component of variance, heritability and genetic advance in per cent of mean for biometrical traits and qualitative traits of four species *viz*., *Spathoglottis albida* Kraenzl., *Spathoglottis gracilis* Rolfe ex Hook.f., *Spathoglottis unguiculata* (Labill.) Rchb.f. and *Spathoglottis plicata* Blume. Results showed that flower colour, the colour and shape of the labellum and bracts were important characteristics used to resolve the morphological difference within *Spathoglottis* species. Biometrical traits *viz*., plant height, length of inflorescence, flower size, capsule size, number of flowers / inflorescence etc. involved significant role in the differentiation of species for utilization in the genetic improvement programme. Breeding method selection can be adopted for the genetic improvement programme since the genetic variability estimate revealed statistically significant high phenotypic coefficient of variation, genotypic coefficient of variation, heritability (broad sense) and genetic advance in per cent of mean for most of these biometrical traits.

**Key words:** Genetic variability, Orchid, *Spathoglottis*, Traits.

**INTRODUCTION**

*Spathoglottis* is very popular among terrestrial orchids because it is attractive, easy to grow and it will flower throughout the year. Therefore, it is being used as a model for orchid studies (Kheawwongjum and Thammasiri, 2008). The vast variability among the species of *Spathoglottis* offers immense opportunity in genetic improvement of this popular crop. It is well acknowledged that variability is prerequisite for determining the efficiency in selection. Moreover, it is also emphasizes the utilization of other breeding programmes where variability is essential for various genetic improvement methods like hybridization, polyploidy breeding, mutation breeding, genetic engineering etc. In crop improvement programme, genetic diversity has been considered as an important factor for obtaining varieties with important desirable characters like disease resistance, earliness or quality of a particular character (Chowdhury, 1975). Of the three types of variability in genetic improvement, genotypic variability is the most important one. The trait with high genetic variability exhibits high genetic inheritance from one generation to next generation. Therefore, the estimation of variability gives an insight about traits which are heritable over generations in crop breeding programmes. Due to the over exploitation and habitat loss the species diversity is dwindling at an alarming rate. Conversely, the popularity of *Spathoglottis* as ornamental ground orchid plants is increasing. In this backdrop, documentation of accurate information on morphological characteristics and genetic variability for important qualitative and quantitative traits of this crop is inevitable for its conservation, genetic improvement as well as sustainable and effective utilisation. To achieve the forgoing objectives, an attempt was made to estimate genetic variability in terms of genetic component of variance such as phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability and genetic advance in per cent of mean for the important biometrical traits involved in the inheritance as well as variation involved in the qualitative traits of four wild *Spathoglottis* species.

**MATERIALS AND METHODS**

The experiment was conducted during 2016-17 and 2017-18 at Saraswathy Thangavelu Extension Centre of Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Puthenthope, Thiruvananthapuram and College of Agriculture, Vellayani, Thiruvananthapuram. Four species of wild *Spathoglottis* *viz* *Spathoglottis albida*...
Kraenzl. *Spathoglottis gracilis* Rolfe ex Hook.f., *Spathoglottis unguiculata* (Labill.) Rchb.f. and *Spathoglottis plicata* Blume (Fig 1A, 2A, 3A and 4A) available at JNTBGRI were selected for the evaluation of genetic variability towards the inheritance of various biometrical traits as well as for the morphological characteristics useful for the genetic improvement in *Spathoglottis* species.

The following methods were adopted for the evaluation of genetic variability in the inheritance of different characters for the adoption of genetic improvement programme in different species of *Spathoglottis* orchids.

The experiment was laid out in completely randomized block design (CRD). Plants of different species were raised in pots under field condition and it was managed following the package of practise recommended by KAU in 2013. Healthy, vigorous, pest and disease free plants were selected for the evaluation. Observations were recorded frequently in order to study the genetic variability with regard to different traits (Table 1). The mature undehisced green capsules or pods developed were harvested and used for evaluation of capsule morphology and seed morphology.

The variability found in four species were assessed and observations with regard to different traits were taken and data were subjected to the statistical analysis for the estimation of genetic variability parameters in order to study the inheritance pattern of different characters for the genetic improvement in *Spathoglottis* (Panse and Sukhatme, 1967). Statistical analysis was carried out to compute the genotypic and phenotypic coefficient of variation, heritability (broad sense) and genetic advance in per cent of mean (GCV, PCV, H2 and GA) on morphological data for the important quantitative characters as per the method suggested by Singh and Chaudhary (1985). Broad-sense heritability (H2) was calculated as the ratio of the genotypic variance to the phenotypic variance using the formula proposed by Allard (1960).

![Fig 1: Floral characters of the selected species.](image)

*Spathoglottis albida*: 1A Habit, 1B Flower colour and floral parts, 1C Mature pod, 1D Seeds.
*Spathoglottis gracilis*: 2A Habit, 2B Flower colour and floral parts, 2C Mature pod, 2D Seeds.
*Spathoglottis unguiculata*: 3A Habit, 3B Flower colour and floral parts, 3C Mature pod, 3D Seeds.
*Spathoglottis plicata*: 4A Habit, 4B Flower colour and floral parts, 4C Mature pod, 4D Seeds.

a. Bract, b. Sepal, c. Petal, d. Labellum, e. Pedicel.
Cytology: Anthers and root tips of the four selected species were collected and used for the observation of chromosome shape and number as per the method suggested by Shindo and Kamemoto (1963). Actively growing immature root tips were excised from the plants and washed thoroughly in tap water to remove soil and other particles adhered to the root portion. These were dipped in 0.1% hydrochloric acid for 15 minutes and then transferred into 1:1 mixture of 70% ethyl alcohol and glacial acetic acid. These were hydrolyzed in same mixture for 5 minutes and squashed and stained in aceto-carmine for 2 minutes and under microscopes observation was taken (Fig 2).

RESULTS AND DISCUSSION

Analysis of variance (Table 2) revealed statistically significant differences for various traits in four species. Mean performance (Table 1) of different traits in four species showed wide variation. *Spathoglottis unguiculata* recorded maximum plant height, bract length, labellum length, labellum width and length of pedicel but minimum bract width. *Spathoglottis albida* recorded maximum spread of the plant, number of leaves, length of leaf, width of leaf, length of inflorescence, number of flowers per inflorescence, capsule length and capsule girth but minimum plant height, flower width, bract width, labellum length and labellum width. *Spathoglottis gracilis* recorded maximum flower length, flower width, sepal length, sepal width, petal length, petal width and labellum length but minimum spread of the plant, number of leaves, length of the leaf, length of the inflorescence, number of flowers per inflorescence, capsule length, capsule girth, bract length, bract width and length of pedicel. *Spathoglottis plicata* recorded maximum bract width only but minimum width of leaf, flower length, sepal length, sepal width, petal length, petal width, petal length and labellum length. Mean performance as well as analysis of variance for various traits influencing the differentiation of *Spathoglottis* species depicted statistical significance and hence it implies that *Spathoglottis* species is highly amenable for genetic improvement.

Genetic components of variance: Variance analysis revealed statistically significant differences among four species. Coefficient of variation such as phenotypic and genotypic variations, heritability estimates (broad sense) and expected genetic advance in per cent of mean (Table 3) revealed that genetic component of variance such as phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were high for most of the
Fig 3: Pollen viability and pollen germination percentage in four *Spathoglottis* species.

| Character                  | *Spathoglottis albida* | *Spathoglottis gracilis* | *Spathoglottis unguiculata* | *Spathoglottis plicata* |
|----------------------------|------------------------|--------------------------|-----------------------------|------------------------|
| Plant height (cm)          | 38.9                   | 49.04                    | 63.75                       | 61.24                  |
| Spread of the plant (cm)   | **105.97**             | 46.7                     | 84.28                       | 80.51                  |
| Number of leaves per plant | 6.45                   | 3.38                     | 4.57                        | 4.93                   |
| Length of the leaf (cm)    | **78.28**              | 37.38                    | 59.02                       | 55.41                  |
| Width of the leaf (cm)     | 7.54                   | 4.25                     | 6.6                         | 3.9                    |
| Length of the inflorescence (cm) | **90.38**         | 43.17                    | 68.75                       | 65.58                  |
| Number of flowers per inflorescence | 58.33                | 10.67                    | 29.5                        | 40.67                  |
| Flower length (cm)         | 4.65                   | 5.53                     | 4.45                        | 4.38                   |
| Flower width (cm)          | 4.65                   | 5.8                      | 5.03                        | 4.72                   |
| Bract length (cm)          | 1.8                    | 1.0                      | **2.0**                     | 1.5                    |
| Bract width (cm)           | 1.0                    | 1.0                      | 1.0                         | **1.1**                |
| Sepal length (cm)          | 2.2                    | **3.5**                  | 2.0                         | 1.9                    |
| Sepal width (cm)           | 1.1                    | **1.9**                  | 1.1                         | 1.0                    |
| Petal length (cm)          | 2.1                    | **3.1**                  | 2.0                         | 1.9                    |
| Petal width (cm)           | 1.3                    | **1.9**                  | 1.5                         | 1.2                    |
| Labellum length (cm)       | 1.9                    | **2.0**                  | **2.0**                     | 1.9                    |
| Labellum lobe width (cm)   | 0.7                    | 1.2                      | 1.3                         | 1.0                    |
| Length of pedicel (cm)     | 4.6                    | 3.0                      | **5.0**                     | 3.5                    |
| Capsule length (cm)        | **6.0**                | 4.92                     | 5.89                        | 5.13                   |
| Capsule girth (cm)         | **2.87**               | 2.42                     | 2.71                        | 2.63                   |

traits analysed. A wide range of variation was observed for all the characters except for capsule girth. Moreover heritability in broad sense and genetic advance in per cent of mean were also high for plant height, spread of the plant, number of leaves per plant, length of leaf, width of leaf, length of inflorescence, number of flowers per inflorescence, flower size (flower length, flower width) and capsule length. But for capsule girth analysis of variance revealed statistically insignificant difference among four species. Genetic component of variance recorded high GCV and PCV and it implies that the influence of environment is nil / negligible. Moreover heritability in broad sense and genetic advance in per cent of mean were also high. It revealed the influence of additive gene effects and hence selection is effective for the genetic improvement of these traits. But capsule girth exhibited low GCV, PCV, heritability and genetic advance and therefore selection may not be effective because environmental influence will also play significant role in the expression of this trait. PCV were slightly higher than the GCV for all the traits studied. This indicated the presence of environmental influence to some degree in the phenotypic expression of the characters. The result was in corroborated with the findings of Sultana (2003), Faroque (2003) and Roychowdhury *et al.* (2011). High heritability (broad sense) estimates were observed for all these traits as reported earlier by Moniruzzaman *et al.* (2012).

**Variation in qualitative characters:** The variation in flower colour, bract shape and colour, capsule shape and colour is presented in (Fig 1, 1B, 2B, 3B and 4B). *Spathoglottis albida* possessed broadly ovate bracts with concave depression, acuminate tip, white coloured with green tinge at the tip of the lobe and white flowers. The flowers of *Spathoglottis*...
gracilis were pale yellow, midlobe claw of the labellum was wider, spathulate with reddish spots, bract apex obtuse and green coloured. Bracts broadly ovate with concave depression, acuminate and light purple coloured with white tinge at the tip of the lobe in Spathoglottis unguiculata and flowers were dark purple. In Spathoglottis plicata oblong-ovate, acuminate, slightly keeled and light purple coloured bracts and flowers were light purple. Bract shape, colour and flower colour were different in these four species and these are important traits determining the classification of different species in orchids especially in Spathoglottis.

Karyomorphology-Karyotype size, shape and number varied among four species. Karyotype number vary from 2n = 16 to 2n = 40 and in Spathoglottis gracilis aneuploidy level variation was noticed (Fig 2-1B, 2B, 3B and 4B). In other species, the observed chromosome number was S. albida 2n = 32 (Fig 1-1B), S. unguiculata 2n =40 (Fig 2-3B) and S. plicata 2n = 32 (Fig 2-4B). According to Sharma et al. (2010) three species of Cymbidium showed distinct inter-specific variation in the arm ratio of few homologous pairs in the chromosome complements. Similarly, in S. plicata different chromosome number was reported by various authors, for example 2n = 16 (Ramesh and Ranganathan, 2008), 2n = 20 (Abraham and Vatsala, 1981) and 2n = 40 (Vijayakumar and Subramanian, 1994). According to Tatik Chikmawati (2013) karyotype variation may be found among S. plicata variants and in karyotype analyses each and every species has a distinct karyotype. Therefore, karyotype alterations may also play important role in speciation along with aneuploidy.

Pollen viability as well as pollen grain germination percentage evaluation was carried out to ascertain capsule development with viable seeds (Fig 2 - 1C, 2C, 3C and 4C). At shedding, the pollen grains were two-celled and the tetrad of pollen grains was enclosed in thick walls. The mature pollen grains were irregular or polygonal in shape and appeared in tightly packed tetrads. Tests of pollen viability among four species revealed that Spathoglottis unguiculata and Spathoglottis albida recorded 100 percent whereas Spathoglottis plicata and Spathoglottis gracilis recorded 80 percent and 70 percent respectively. Similarly, estimation of pollen germination percentage showed that 100 % of pollen germination in Spathoglottis unguiculata and Spathoglottis albida meanwhile it was 85 percent in Spathoglottis plicata and 75 percent in Spathoglottis gracilis (Fig 3). Pollen viability has been investigated in terms of its contribution to incompatibility and fertility studies or crop improvement and breeding projects (Stone et al., 1995). Additionally, it was reported that genotypic, phenotypic and environmental factors play vital role in determining the duration of pollen viability (Dafni and Firmage, 2000). Pollen grains are surrounded by pollen kit or elastoviscin (Schill and Wolter, 1986; Pacini, 1997) and commonly packaged into dispersal units (Pacini and Hesse, 2002) and hence viability will be more. Present findings revealed 100% of

Table 2: ANOVA for traits among Spathoglottis species.

| Source of Variation | Plant height (cm) | Spread of the plant (cm) | No. of leaves/ plant | Length of the leaf (cm) | Width of the leaf (cm) | Length of the inflorescence (cm) | No. of flowers/inflorescence | Flower length (cm) | Flower width (cm) | Capsule length (cm) | Capsule girth (cm) |
|---------------------|------------------|-------------------------|--------------------|------------------------|------------------------|--------------------------------|-----------------------|------------------|------------------|-------------------|------------------|
| df                  | 6                | 21.06                   | 0.86               | 0.66                   | 0.53                   | 0.00                           | 0.00                   | 0.00             | 0.00             | 0.00              | 0.00             |
| MSE                 | 111.135          | 261.766                 | 0.85               | 0.69                   | 0.53                   | 0.00                           | 0.00                   | 0.00             | 0.00             | 0.00              | 0.00             |
| CD                  | 21.06            | 32.55                   | 0.58               | 1.67                   | 14.902                 | 0.00                           | 0.00                   | 0.00             | 0.00             | 0.00              | 0.23             |

Table 3: Genetic variability –PCV, GCV, Heritability and Genetic advance in per cent of mean for various traits among Spathoglottis species.

| Characters | Genetic variability | Heritability | Genetic advance percent |
|------------|---------------------|--------------|-------------------------|
|            | PCV                 | GCV          |                         |
| Plant height (cm) | 31.09             | 23.23        | 0.55                    | 35.23                   |
| Spread of the plant (cm) | 33.68             | 26.92        | 0.64                    | 43.71                   |
| Shoot girth (cm) | 22.87             | 19.93        | 0.75                    | 35.34                   |
| No. of leaves/plant | 26.61             | 25.89        | 0.95                    | 51.94                   |
| Length of the leaf (cm) | 32.83             | 27.12        | 0.68                    | 45.99                   |
| Width of leaf (cm) | 27.91             | 22.55        | 0.65                    | 37.33                   |
| Length of the inflorescence (cm) | 30.25             | 28.12        | 0.86                    | 53.58                   |
| No. of flowers/inflorescence | 62.14             | 54.96        | 0.78                    | 99.86                   |
| Flower length(cm) | 11.63             | 10.95        | 0.89                    | 21.09                   |
| Flower width(cm) | 10.90             | 10.20        | 0.88                    | 19.54                   |
| Capsule length(cm) | 10.31             | 9.52         | 0.85                    | 18.04                   |
| Capsule girth(cm) | 16.32             | 7.68         | -0.22                   | -7.39                   |

GCV = Genotypic coefficients of variation, PCV = Phenotypic coefficients of variation.
pollen viability as well as pollen germination in *Spathoglottis albida* and *Spathoglottis unguiculata*.

Seed morphology observed through the microscope (Fig 1-1D, 2D, 3D, 4D) showed that each seed consists of a tiny round or ovate embryo surrounded by a translucent coat. The micromorphology of the seeds revealed that they are minute, dust-like and fusiform in shape. Have an aperture in the posterior. The testa cells were longitudinaly oriented, tetragonal or polygonal in outline with no intercellular spaces between them. Inner to seed coat there was a golden brown embryo occupied at the central area, conforming to the widest zone of the seed forming an ovoid shape. Shape and size of embryo, and shape and colour of seed coat varied among four species. Orchid seeds are very minute in size and non-endospermic (Semiarti et al., 2014). Therefore, it needs specific nutritional and environmental conditions (Arditti et al., 1990) for germination. Orchid seeds vary based on their size, the shape of the seed coat and their colour, but all are very small (Holttum, 1957).

Based on this study selection of species with desirable qualitative and quantitative attributes play significant role in the genetic improvement because almost all traits evaluated exhibited high heritability as well as high genetic advance and also high GCV and PCV revealed very little effect of environment. These four species also exhibited high variation for different oleigogenic traits such as bract length, colour, shape, flower colour, capsule colour, capsule shape, seed shape, seed viability, karyotype morphology etc.

**CONCLUSION**

*Spathoglottis* species viz., *Spathoglottis albida*, *Spathoglottis gracilis*, *Spathoglottis unguiculata* and *Spathoglottis plicata* exhibited wide variation in morphology and hence study of genetic variability is essential for the development of genetically improved types. The present findings revealed the existence of high genetic variability in terms of GCV, PCV, heritability and genetic advance for most of the qualitative and quantitative traits including karyotype analysis. Therefore, the results concluded that for the genetic improvement of *Spathoglottis* species breeding method such as selection will be effective because of the existence of additive genetic effect as per the result obtained in the genetic analysis and would be effective for plant breeders in developing new orchid varieties in future. Determination of species status helps to incorporate valuable genes for the genetic improvement.

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