# CHAPTER THREE METHODOLOGY

This chapter describes: (1) the subjects, (2) the materials, (3) the procedures used in the collection and analysis of data, and (4) the data analysis.

## 3.1 SUBJECTS

In this trial, a new variety or the so-called candidate variety of the orchid, *Dendrobium* hybrid (Dangphimon variety) was tested to determine if it was unique when cpmpared to the existing variety or the so-called reference variety, Dangphiiriya. Hence, the sample in this trial comprised two varieties of *Dendrobium*.

#### **3.2 MATERIALS**

The purpose of this section is to describe the materials and technique employed in the gathering of the data.

3.2.1 Selection of Samples

(1) 20 plants of candidate variety were randomly taken from the 2year breed of *Dendrobium* plants.

(2) A random selection was made of 20 plants of reference variety at the same age as the candidate variety and chosen from the same species, or the most similar morphologically to the candidate variety and grown in Thailand at the time of trial was also random taken.

3.2.2 Allocation of Samples and Conditions

Both the candidate and reference varieties allocated to the trial group using sample randomization were grown in the same location. The test included a total of 40 plants divided into 2 replications of each variety by placing them in 4 parallel rows, (Table 3). A shade providing 70 % of coverage was applied. The plants were propagated from tissue culture or cuttings and potted with coconut bark. Nutrition, pest and disease control was applied as required on the basis of good agricultural practice.

### *Table 3.* Test plot design

Number of rows	4
Row length	2m
Row to row distance	40cm
Plant to plant distance	40cm
Number of replications	10

## 3.3 **PROCEDURE**

### 3.3.1 Research Design

According to Kumar, 1999, a comparative experimental design was used in this trial. Both quantitative and qualitative values were observed during the test period. The trial was conducted at the breeder's premise in Samut Sakon province from the beginning of October, 2005. As prescribed in the Plant Variety Protection Act, the *Dendrobium* species required one growing seasons of comparative test and trial, which was approximately three months from the date that the samples started to be taken.

## 3.3.2 Data Collection

#### 3.3.2.1 Variables

The data was collected once ("กรมวิชาการเกษตร", 2546, น 63-39)

during the harvesting period (2/3 of the first inflorescence bloom). In order to assess DUS, the table of characteristics of the *Dedrobium* varieties was formulated as a

guideline. The characteristics were marked with a (\*) symbol were used to indicate all the varieties and always incorporated in the table of characteristics, except when the state of expression of a preceding characteristic or regional environmental conditions render this impossible. The characteristics were marked with a (+) symbol to indicate that there was further explanation or an illustration in the Appendix A.

In DUS trials, many of characters were expressed in terms of measurements such as plant height, leaf length, leaf width, flower diameter etc. In this trial, both quantitative and qualitative values were observed. The breeder claimed that the shape, size, and color of flower were novelty. Therefore, flower characteristics were dependent variables collected to satisfy the main objective. On the other hand, other characteristics were dependent variables as well but they were collected to satisfy the sub objectives. Chemicals being applied during the test, however, were considered as independent factors and will not be taken into account.

#### 3.3.2.2 Measurement

Distinctness, uniformity and stability were established individually for each plant. Observations and measurements were made of 40 plants according to the Act. The most common method of recording the characteristics in this trial were:

(a) single plant recording (e.g. length and width measurements and counts)

- (b) plot scoring
- (c) single plant scoring
- (d) comparative recording (usually visual assessments)

The variables recorded were listed in the guideline of the appendix. Moreover, photographs were taken during observation, as they were the most crucial tools to evaluate how the candidate variety was distinct from the reference varieties.

#### 3.3.2.3 Methods and Observations

(a) For the assessments of distinctness:

All observations for the assessment of distinctness and stability were made of 20 plants randomly selected, or of the parts taken from each of those plants. The distinctness was determined by the clarification of difference between a specific characteristic of the candidate variety and that of the example variety. As for the qualitative characteristics (observation: color, shape), the candidate variety and the example variety were considered as distinct when a specific characteristic of each variety yielded 2 different descriptions. As for the quantitative characteristics (counting: size), the substantial distinctness between the candidate variety and that of the example variety were determined based on the value of the Least Significant Difference (LSD) at the minimum of 95%.

(b) For the assessment of uniformity:

The main method of assessment of uniformity of the candidate variety was based on the calculation of ratio between off-type plants and the plant population on the observation plot. The proportion of standard sample against off-type plants was at a minimum of 5%, the minimum reliable ratio then having to be 95%. In the case of a sample size of 20 plants (of 2 replications), the maximum number off-types allowed was 2 plants.

(c) For the assessment of stability:

The stability of the variety was assessed indirectly through the assessment of distinctness and uniformity. If the assessment data of the experimental seasons were the same or reaching a minimum distances of 95% the candidate was regarded as stable.

## 3.4 DATA ANALYSIS

#### 3.4.1 Qualitative Data

68 out of 88 characteristics from the table of characteristics are considered as qualitative data assessed by the visual observation of 10 plants of each variety

#### 3.4.2 Quantitative Data

29 out of 88 characteristics from the table of characteristics are considered as quantitative data assessed by the measurement of the mean value of each characteristic among the 10 plants of each variety

## 3.4.3 Image Data

Pictures of each characteristic may be used to judge the distinctness in some cases. Therefore, pictures of major characteristics such as the flower, leaf, and pseudobulb were taken during the examination.

Because there were continuous variables expected to follow normal distribution with  $\mu$  mean and  $\sigma^2$  variance, a T-test was used for statistical analysis. However, in the case of flower color groups, the distinctness was established by classifying the individual variety using the Royal Horticultural Society (RHS) color chart to identify their colors.

In summary, this chapter has shown the methods and procedures of study that would lead to the output and outcome of the study. In the next chapter, the assessments and observations of the test will be presented.