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# Abnormal and cytoplasmic connection of guard cells of stomata of leaf of six species of the monocots

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**Abstract:** The present investigation describes abnormal stomata and cytoplasmic connections between guard cells of neighboring stomata in mature leaf of six species of the monocots. The study is made on leaves of *Amaryllis reticulata* L. Her., *Narcissus pseudonarcissus* L., *Iris langport* Wern., *Crocus sativus* L., *Ixiolirion tataricum* (Pall.) and *Allium cepa* L. anomocytic stomata type was observed in all species. Several stomata abnormally include single guard cells, aborted guard cells arrested developments and cytoplasmic connection between guard cells of neighboring stomata was common to all species except in *Allium cepa* wasn't observed cytoplasmic connection.

**Keywords:** Narcissus, Iris, Crocus, Ixiolirion, Allium, Cytoplasmic connection

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## 1. Introduction

Stomata are composed of a pair of guard cells that located on the abaxial and adaxial sides of plant leaves [1]. The stomata of *Bougainvillea spectabilis* were anomocytic on abnormal leaf. The guard cells were kidney-shaped on normal and abnormal leaf [2]. The stomata were observed in the seeds, primary root, leaf and other parts of plants [3,4]. Stomata play an important role in plant innate immunity [5]. Some researchers seek the possible track of plant genetic variation according to studying leaf epidermal stomatal morphology [6,7]. In addition, leaf epidermis stomatal morphology has significance in taxonomy [8]. The stomatal complex of coconut is characterized by the presence of two guard cells and four subsidiary cells, two of which are roundish and the other two are lateral side to the guard cells [9]. Abnormal stomatal patterning or "stomatal cluster" as it is known, has been reported successively in certain species of the family *Begoniaceae* (*Begonia cavaleriei* L.), *Crassulaceae* (*Sedum lineare* and *S. aizoon*), *Sonneratiaceae* (*Sonneratia alba* J. Smith), and *Moraceae* (Some species of *Ficus*) [10]. This distribution is quite different from the normal stomatal patterns [11]. Such stomatal clusters were reported in more than 60 species in the family *Gymnosperm*, *Dicots*, and *Monocots* [12]. Two types of stomatal clusters have found among plants: type A clusters have 2 (or more) stomata placed in direct contact (without

intervening epidermal cells between neighboring guard cells), such as: *Alysicarpus procumbens* Schindl from *Papilionaceae* [13], *Sonneratiaceae*, *Ginkgo biloba* [14] and *Annonaceae* [15]. While type B clusters are formed by groups of stomata that do not contact with each other (they are separated by the subsidiary cells). Those plants are *Crassulaceae* (*S. lineare*) [16], *Himantandra parvifolia* Bak [17]. These two types of clusters are still not well classified: the term "stomatal cluster" has been used for both types in many studies [18]. Therefore, a clear definition and classification must be redrawn.

Foliar epidermis of nineteen species of *Amaryllidaceae*, has been studied. Various types of stomatal abnormalities viz contiguous stomata, interstomatal cytoplasmic connections etc., are observed in the different species [19]. Leaves of some plants contain normal stomatal patterning and the two types of clusters. These used the classical methods which were developed to assess stomatal distribution in ecological studies [20] to evaluate the difference between these two stomatal cluster types.

## 2. Materials and Methods

Isolation of leaf epidermal layers in the present study, stomatal complex was studied by peeling method of leaves. Mature leaves were used to study the dermal characters according to the methods suggested for orchids by Williams [21]. The epiderm peels were obtained from mature leaves of

*Amaryllis reticulata* L. Her., *Narcissus pseudonarcissus* L., *Iris langport* Wern., *Crocus sativus* L., *Ixiolirion tataricum* (Pall.) and *Allium cepa* L. Epidermal peels were stained by 0.1% aqueous methylene blue, 0.1%, aqueous nutrient red, 0.1% aqueous rodamine and 1% aqueous safranin for about 3-5 min and mounted in 5% glycerine. A portion of each macerated cuticle was taken for microscopic studies. Observations were made on the microscope to determine stomatal complex types and normality and abnormality stomatal frequency include neighbors' stomata, stomata without guard cells, cytoplasmic connection stomata, un-connection stomata degenerated guard cells frequency. determination of frequency of stomatal complex types using 10 fields of view at 10X and 40X objective as a quadrat, the number of subsidiary cells per stoma was noted to determine the types of stomatal complex present in each specimen. Frequency of each stomatal complex type was expressed as % occurrence of each stomatal complex type based on occurrences of stomatal complex types [22,23]. The slides were observed in Trinocular microscope and photographs were taken. Results were common for all used staining methods.

### 3. Results and Discussion

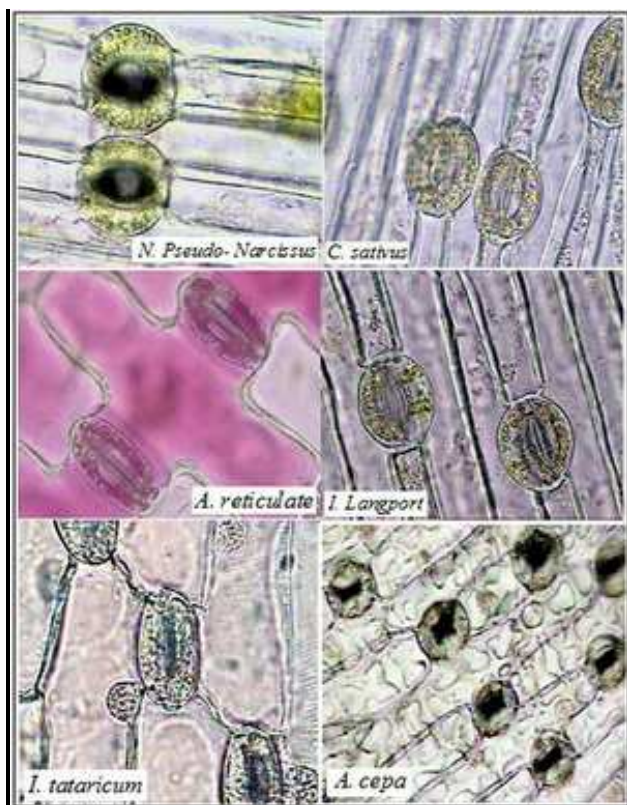


Figure 1. The stomata of all species were anomocytic type and Epidermal cell walls were straight in *N. pseudonarcissus*, *I. langport*, *C. sativus*, *I. tataricum* and *A. cepa*, Only in *A. reticulata* was cross shape.

The stomata of all species were anomocytic type (Figs 1), and their characteristics are shown in Table 1. Epidermal cell walls were observed to be straight in all species (Fig 1), Only in *A. reticulata* was cross shape (Fig 1). Three major types of

stomata such as paracytic, tetracytic and anomocytic type present in monocotyledonous plants [24]. *Orchidaceae* is one of the largest families among monocots. Epidermal studies of orchids showed anomocytic or paracytic stomata are very frequent [25]. The stomata foliar epidermises of nineteen species of *Amaryllidaceae* were anomocytic. Various types of stomatal abnormality contiguous stomata, interstomatal cytoplasmic connections etc., were observed in the different species [19].

In all species stomata abnormally was observed (Tab 1). Several stomata abnormally were include single guard cells in relationship with normal stomata, aborted guard cells, arrested developments and cytoplasmic connection between nearby stomata or between stomata and epidermal cells. The normale stomata of *Narcissus pseudonarcissus* L., was the lowest (61%) but in *Iris langport* Wern. was the highest (98.5%).

Table 1. Percent of normal and some abnormally characters of stomata of *N. pseudonarcissus*, *I. langport*, *C. sativus*, *I. tataricum* and *A. cepa*, and *A. reticulata*

plant	%ns	%obs	%nes	%ccs	%ucs	%dgs	%swgc
<i>A. reticulata</i>	86	14	45	8	37	5	1
<i>N. pseudo narcissus</i>	61	39	30	22	8	15	2
<i>I. langport</i>	98.5	1.5	10	1	9	0	0.5
<i>C. sativus</i>	91	9	14	8	6	0	1
<i>I. tataricum</i>	91	9	41	1	40	5	3
<i>A. cepa</i>	91	9	29	0	29	6	3

ns: normal stomata, obs: obnormal stomata, nes: neighbors stomata, ccs: cytoplasmic connection stomata. %ucs: un connection stomata, %d gs: degenerated guard cells of stomata, %swgc: stomata without guard cells.

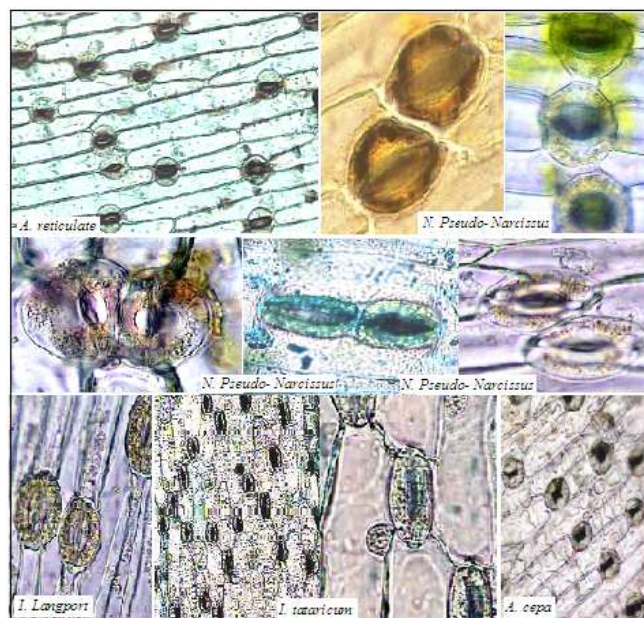


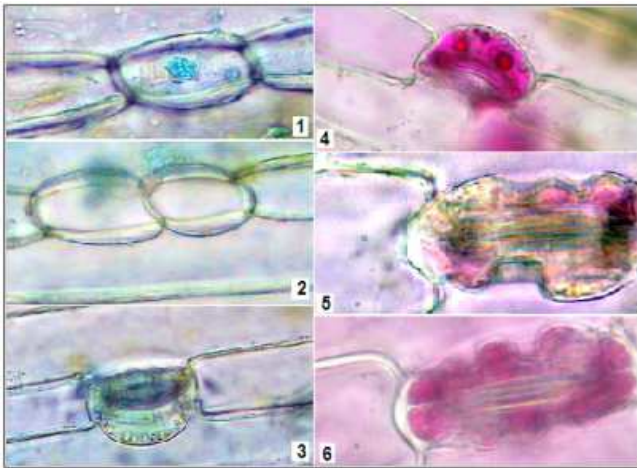
Figure 2. Stomatal clustering in *A. reticulata* and *I. tataricum*, in *I. langport*, *A. cepa* and *N. pseudonarcissus*.

Stomata clustering and poly contiguous stomata clustering were observed in the leaf epidermis of *A. reticulata* and *I. tataricum*, two contiguous stomata in *I. langport*,



laterally poly juxtaposed contiguous stomata clustering in the leaf epidermis of *A. cepa* and laterally two and three contiguous stomata in *N. pseudonarcissus* l. (Fig 2). Stomatal clustering," an abnormal stomatal patterning that is formed by two or more stomata in the leaf epidermis, has been reported in more than 60 species of terrestrial plants [26].

2- The stomata abnormalities include the stomata with one or two single guard cells were observed in *N. pseudonarcissus* and the degenerated guard cells in *A. reticulate* and *N. pseudonarcissus* (Fig.3). In the first type, either one of the guard cells was transformed into a large thin-walled cell or the two neighboring cells by arrested developments. In the latter case the stomata pore is flanked by a single guard cell on one side and two ordinary epidermal cells on the other. The second type is caused by the one or two guard cells heteromorphy by cells degenerated and crushed. Seven types of aberrant and five types of normal stomata of the seedlings of 21 species of *Solanaceae* are reported. Anomalous stomata were include single guard cell with or without pore; cytoplasmic connection between nearby stomata suggesting their physiological relation; aborted guard cells; and arrested development[27]. The stomatal abnormalities like juxtaposed contiguous stomata and cytoplasmic strands were observed in *Asparagus flagellaris* and *Asparagus africanus* respectively. The juxtaposed contiguous stomatal abnormalities were between 3 guard cells while the strand was between 2, 3,4,5,6 stomata [28].

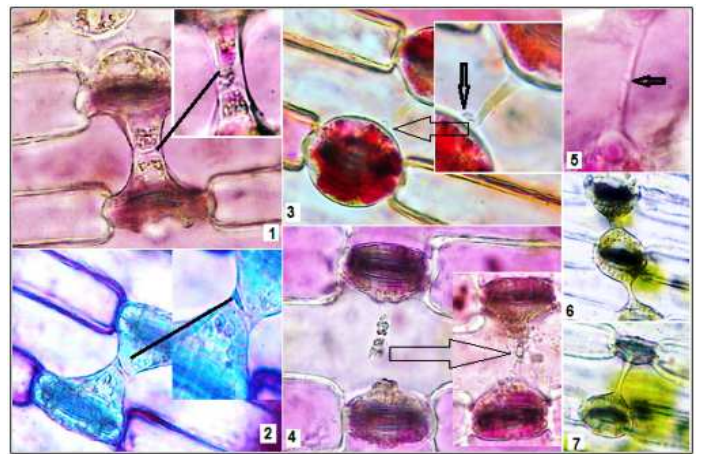


**Figure 3.** The stomata abnormalities: 1,2 ,3- one or two single guard cells in *N. pseudonarcissus* and 4,5,6- the degenerated stomata in *A. reticulate*, *N. pseudonarcissus*

3- Cytoplasmic strands and connection were observed in all species except *A. cepa*. The highest cytoplasmic strands between guard cells were observed in *N. pseudonarcissus* (22%), *A. reticulate* (8%) and *C. sativus* (8%) respectively (Tab. 1 and Fig. 4). Cytoplasmic connection were absolved between 2, 3, 4, 5 and 6 neighboring stomata of *N. pseudonarcissus*, *A. reticulate* (Fig. 4).

Cytoplasmic connection between nearby stomata was the a protuberance arises from one of the guard cells of a nearby stomata(Fig. 4:3, 4, 5). The protuberances grow

more and more, ultimately meeting and fusing in the center, their fusion wall disintegrated, and a communication channel is formed between two nearby stomata (Fig. 5). This tubular connection appears like a conjugation tube between two algal filaments. These guard cells have a cytoplasmic connection which is secondarily developed. The cytoplasmic connection between nearby placed stomata and sometimes between one of the guard cells and an epidermal cell were also observed. This indicates that there is some physiological connection between the nearby stomata and guard cell and an epidermal cell. Dahnel observed that they were connected to the relationship of environment and gene structure [29], while Gopal and Shah suggested that contiguous stomatal abnormality are produced due to budding [30].



**Figure 4.** Cytoplasmic strand between two stomata in, *A. reticulate* (1,3,4), *N. pseudonarcissus*(2,5,6,7).



**Figure 5.** Cytoplasmic strand between two guard cells of two neighboring stomata of *N. pseudonarcissus*

4- Division of guard cell(s) were observed in leafs of *Crocus sativus* L. (Fig. 6). The guard cells were arised by equal division due to mitosis of the guard mother cells. Anomocytic stomata were arised directly from the

meristemoid by a straight division and without cutting off any subsidiary cells. At its origin and in early stages of growth, the leaf primordium consists of an undifferentiated mass of contiguous cells without intercellular spaces. The homogeneous mass of cells is organized into layers that soon differentiate and acquire specialized functions. Since plant cells are locked into position by their walls, differentiation takes place without cell movement. However, cells do undergo enlargement particular to their identity and effectively move away from neighbouring cells. The distribution of *Crocus sativus* stomatal initials on the abaxial blade surface is irregular, but mature stomata are regularly ordered because some initials cease to develop (Fig. 6). Approximately 17% of undifferentiated epidermal cells become stomatal initials and about 3% of the initials arrest early in development. Arrest of developing stomata is a common phenomenon in monocots (wheat, corn, Acorus, Brocchinia, Setcreasea, Chlorophytum, Croxdale) [31].

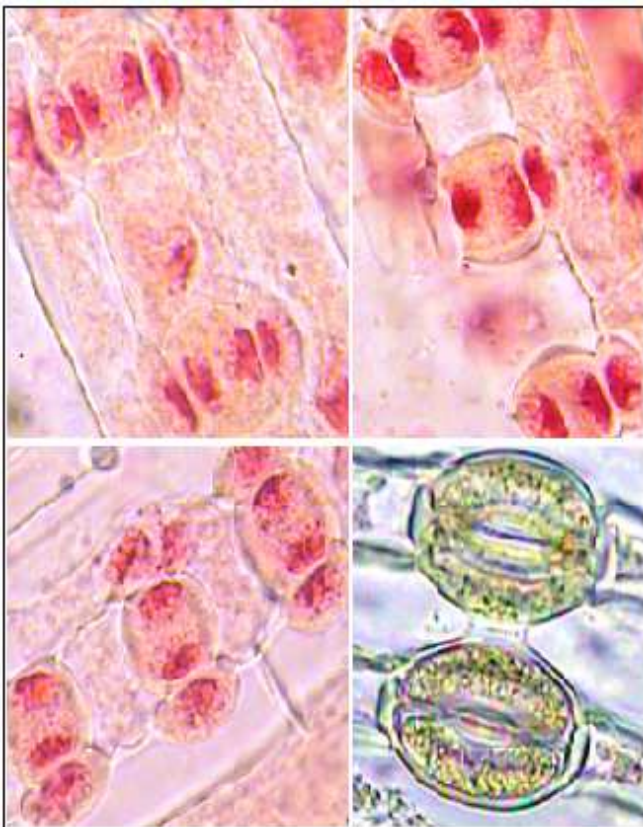


Figure 6. Division of guard cell(s) of leaves of *Crocus sativus* L.

#### 4. Conclusion

The stomata of all species include *Amaryllis reticulata* L. Her., *Narcissus pseudonarcissus* L., *Iris langport* Wern., *Crocus sativus* L., *Ixiolirion tataricum* (Pall.) and *Allium cepa* L. were anomocytic type. In all species stomata abnormally was observed. Several stomata abnormally were include single guard cells in relationship with normal stomata, aborted guard cells, arrested developments and cytoplasmic connection between

nearby stomata or between stomata and epidermal cells. Stomata clustering and poly contiguous stomata clustering were observed in the leaf epidermis of *A. reticulata* and *I. tataricum*, two contiguous stomata in *I. langport*, laterally poly juxtaposed contiguous stomata clustering in the leaf epidermis of *A. cepa* and laterally two and three contiguous stomata in *N. pseudonarcissus*. Cytoplasmic strands function as translocation channels, and this therefore shows that the guard cells are not physiologically isolated from other epidermal cells. Cytoplasmic strands and connection was observed between guard cells of mature leaves of *N. pseudonarcissus*, *A. reticulata*, *C. sativus*, *I. tataricum* and *I. langport*.

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