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Relationship Analysis of Phenology, Stress Tolerance, and Mean Productivity in Wilt and Cold Stressed Chickpea (*Cicer arietinum* L.) Following Synthetic PGRs Application

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

To understand the phenotypic response to mitigate stress tolerance for achieving maximum mean productivity, a comparative study of four synthetic PGRs—Abscisic Acid (ABA), Naphthyl Acetic Acid (NAA), Salicylic Acid (SA), and Fusaric Acid (FA)—was conducted as a pot experiment

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against artificially inoculated Fusarium oxysporum (a wilt pathogen) and cold exposure in four chickpea varieties. Additionally, the relationship between traits and PGRS application was looked at to evaluate their role in the stress tolerance mechanism. The results showed that ABA at 5 and 2 ppm was effective in delaying flowering, therefore extending the vegetative development phase in plants. In this way, flowering promotes stress tolerance while evading the damaging impacts of wilting and cold. This resulted in a lower percentage of wilt and a reduced incidence of cold compared to all other treatments, which raised mean productivity. The use of ABA at 5 and 2 ppm has been shown to positively correlate with both the prolongation of vegetative development and the delay of flowering. But fusaric acid (FA), a fungal toxin, is what caused early flowering, which allowed the flower to coincide with the development of wilt and cold. The occurrence of wilt at seedling and cold during flowering accelerated the incidence of wilt and cold, which led to a reduced mean productivity after giving fusaric acid (FA) @ 10 and 20 ppm. According to the investigation, the application of fusaric acid (FA) at 10 and 20 ppm was found to be positively and highly correlated with an increase in the incidence of wilt and cold and consequently negatively correlated with the mechanism possessed by ABA at 5 and 2 ppm.

Keywords: Phenology; Abscisic Acid (ABA); Fusaric Acid (FA); correlation; stress tolerance; mean productivity.

1. INTRODUCTION

After field peas and dry beans, chickpeas (Cicer arietinum L.) are the most significant winter-season grain legume crop globally. It is also a crucial part of crop rotation. Currently, chickpeas are grown on around 13.8 million hectares of land globally, yielding 9.83 q ha^{-1} and producing 13.65 million tons. Grown on around 10.5 million hectares, it yields 11.15 million tons and 10.56 q ha^{-1} productivity in India. Madhya Pradesh has 3.5 million hectares of total chickpea land, with a productivity of 10.82 q ha^{-1} and a production of 4.59 million tons [1]. Protein (220 g kg^{-1}), total carbohydrates (670 g kg^{-1}), starch (470 g kg^{-1}), and fat (50 g kg^{-1}) are all present in significant amounts in chickpea seeds. They thus have a major impact on the human dietary system [2]. In most chickpea-growing regions of the world, Fusarium wilt, caused by Fusarium Oxysporum, significantly reduces chickpea yield. Fusarium wilt is regarded as a significant foreign disease that can cause annual chickpea output losses ranging from 10% to 15%, with the potential to cause the crop to completely collapse under certain circumstances [3]. Furthermore, chickpeas exhibit poor pod set, sterile pods, abortion of pods, poor flower set, and abortion of flowers when they are subjected to cold stress during the reproductive phase, particularly during flowering and pod formation [4]. The following can be used to categorize the many phenological stages of chickpea growth: emergence, flowering, pod initiation, pod set, seed development, and physiological maturity. Since the duration of the reproductive phase and the environmental circumstances that prevail during flowering impact the percentage of fruit set and the eventual yield, flowering is regarded as the key period [5]. Planning, scheduling, and carrying out our farm tasks on time can be made easier with knowledge of the timing and variety of phenological phenomena. With this knowledge, we may also modify our farming practices to maintain our farm's produce in the face of anticipated climate change [6]. Acquiring an understanding of the correlation between phenological events and other qualities is also necessary to manipulate or perform agricultural plant behavior in a way that interests us [7]. Plant hormones—which are primarily organic compounds—modify the developmental pattern and yield response of crops. With the assistance of PGRs, which primarily regulate the defensive responses of plants through antagonistic and synergistic interactions known as signaling crosstalk, plants have evolved sophisticated mechanisms to detect environmental signals and can initiate an ideal reaction to stressful circumstances [8]. Therefore, the present investigations are aimed to studying the relationship between phenology, stress tolerance, and mean productivity in wilt and cold-stressed chickpea (*Cicer arietinum* L.) after the application of different synthetic PGRs.

2. MATERIALS AND METHODS

In the years 2020–2021 and 2021–2022, the research was conducted at the Herbal Garden Department of Plant Physiology, JNKVV, Jabalpur (MP). Four varieties (V1 (JG74, susceptible to wilt), V2 (JG11, susceptible to cold), V3 (RAJAS, resistance to wilt), and V4 (PBG5, tolerant to cold) were used in the factorial completely randomized design of the crop planted in pots. At various concentrations Spraying of PGRs, like Abscisic Acid (ABA), Naphthyl Acetic Acid (NAA), Salicylic Acid (SA), and Fusaric Acid (FA), was performed two times, once during the early seedling stage and again at the flower initiation stage.

2.1 Method of Wilt and Cold Application

When crops reach the 20 DAS stage, inoculation of wilt in pots was performed according to the method used by Thakur et al. [9], and in accordance with the treatment schedule, the first foliar spray of various PGRs was applied when plants showed signs of wilting. At the floral initiation stage, cold treatment was also artificially done as per the method used by Thakur et al. [9], and after applying wilt and cold treatments, a second spraying of the PGRs was done.

2.2 Phonological Studies

Throughout all crop seasons, visual observations were made every two days to track the crop's phenological development from the beginning of flowering until maturity.

2.3 Wilt Incidence (%)

After applying wilt inoculum in a pot, the number of plants that displayed noticeable signs such as wilting, chlorosis, and browning of the vascular system was counted, and the Mayee and Datar [10] methodology was used to determine the percentage wilt incidence.

2.4 Cold Incidence (%)

It was calculated using the method proposed by Mayee and Datar [10], with appropriate modifications.

2.5 Mean Productivity

Mean Productivity was calculated by using formula -

$$MP = (Ypi + Ysi) / 2$$

Ysi and Ypi are the mean grain yields of individual treatments under stressed and non-stressed conditions.

2.6 Statistical Analysis

The data were statistically analyzed through a completely randomized design given by Fisher (1955), and comparison of means was performed on the basis of the least significant difference test (LSD) according to the method given by Gopinath et al. [11]. The association between the features we took into consideration in our study and the application of PGRs, as well as their respective contributions to the stress tolerance mechanism for achieving maximum mean productivity was subsequently studied using PCA biplot analysis.

3. RESULTS AND DISCUSSION

From the pooled data **(**Fig. 1) obtained from years (2020-2021 and 2021-2022), it was found that hormonal treatment differed significantly ($p > 0.05$) for days required to flower initiation, completion of flowering, pod initiation, and seed development. Higher number of days required for flowering **(**Fig. 1) (48.39 & 47.20 DAS), complete flower formation (70.40 & 69.95 DAS), pod initiation (79.86 DAS), and seed development (87.35 DAS) were taken under the treatment of ABA @ 5 PPM and ABA @ 2 PPM. The application of ABA delayed flowering, increasing the length of vegetative growth and preventing flowers from being negatively impacted by cold treatment. This prevented flowers from being susceptible to cold shock and lessened its damaging effects. Increased photo assimilated amounts that the crop plants used for their reproductive growth cycle are also attributed to longer vegetative growth durations [12]. A PCA-based biplot analysis (Fig. 2, 3, 4) was used to attribute the relationship between plant modulators and traits that show stress tolerance and boost crop yields under wilt and cold-induced conditions. The PCA-biplot analysis's trait vector angles show how the variables are correlated. When the angle between two trait vectors is less than 90°, it is positively correlated, when it is more than 90°, it is negatively correlated, and when it is equal to 90°, it is correlated independently. The characteristics linked to stress tolerance were distinguished into four categories using the PCA-biplot analysis: strong positive correlations, positive correlations, independent correlations, and negative correlations. The results showed that both treatments had a favorable correlation with the start and completion of flowering and the number of days required for pod initiation and seed development; as a result, the more these features were valued by the plants after these treatments were sprayed, On the other hand, using PCA-biplot (Fig. 2, 3, 4) analysis's a negative correlation was found between ABA @ 5 PPM and ABA @ 2 PPM with fusaric acid treatment @ 20 ppm and 10 ppm. This resulted **(**Fig. 1) in the earliest flowering (40.24 & 41.57 DAS) and complete flower formation (63.28 & 64.83 DAS), as well as a shorter duration of days required for pod initiation and seed development under both of these treatments. One possible explanation is that fusaric acid, a fungal toxin, causes wilt in treated plants, and plants that experience abnormalities from internal or external sources, attempt to complete their life cycle as quickly as possible, which leads to early flowering when fusaric acid is applied [13].

Variety V4 (PBG5) **(**Fig. 1) had a greater number of days needed for flower initiation and flowering completion than variety V1 (JG74), which had the earliest flower initiation and completion times, at 41.55 & 63.17 days, respectively. Similarly, V_4 (PBG5) **(**Fig. 1) required more number of days for pod initiation (79.86 DAS) and seed formations (87.35 DAS) due to its normal growth period, which resulted from its tolerance capacity to nullify adverse impacts imposed by wilt and cold, whereas V1 (JG74) had a requirement of a smaller number of days for pod initiation (69.20 DAS) and seed formations (73.61 DAS), which might be due to changes in the growth period imposed by wilt and cold. Similar findings were also observed by Veeramani and Sendhilvel [14], who discovered that the variety with wilt incidence (17.20%) required significantly less time to reach 50% flowering than the variety with incidence (11.50%), which required more time. Here again, a positive correlation (Fig. 2, 3, 4) is found between phenophase and stress tolerance. The variety that can tolerate stress better, grows normally, or has a longer lifespan. These traits are similar to the trend observed with the hormone.

The number of days needed for physiological maturity (107.78 & 105.53 DAS) and harvestable maturity (119.96 & 116.89 DAS) **(**Fig. 1). increased as a result of treating the plants with ABA @ 5 ppm and ABA @ 2 ppm. Again, both treatments had a positive correlation with both of these traits and achieved a higher value than other treatments. Whereas, due to the negative correlation (Fig. 2, 3, 4) of fusaric acid treatment @ 20 ppm and @ 10 ppm with ABA @ 5 ppm and ABA @ 2 ppm, it took a shorter time to acquire physiological (92.90 & 95.54 DAS) and harvestable maturity (101.70 & 104.50 DAS) **(**Fig. 1). under fusaric acid treatment @ 20 ppm and @ 10 ppm. Our results are consistent with the findings of Qi and Zhang [15], who observed that crop plants reduce their growth period and attempt to complete their life cycle in a shorter amount of time by making the most use of available resources when they experience abnormalities brought on by any internal or external factors. In comparison **(**Fig. 1), genotype V1 (JG74) needed the fewest days (93.50 DAS) and (101.28 DAS) for physiological development (104.01 DAS) and harvestable maturity (115.60 DAS), while genotype V4 (PBG5) required the maximum days **(**Fig. 1). Stress tolerance and susceptibility traits vary among genotypes, and this is reflected in the range of days needed for physiological maturity and harvestable maturity [16]. For physiological development and harvestable maturity, genotype V4 (PBG5) **(**Fig. 1), needs the greatest number of days, while genotype V1 (JG74), which is more sensitive, needs the fewest days.

Pooled data from both years (2020-2021 and 2021-2022) **(**Fig. 1) shows that treatments with FA @ 20 ppm and FA @ 10 ppm exhibited the highest disease incidence of 31.25% and 30.71%, respectively. One well-known fungus toxin that causes chickpea death is fusaric acid [17]. FA's synergistic actions with wilt inoculums caused foliar sprays to accelerate wilting in wilt-contaminated pots, which in turn caused the chickpea crop to die soon. Due to alterations in crop plant structure and functional activities, plants became more susceptible to several kinds of stresses when disease incidence increased under FA @ 20 ppm and FA @ 10 ppm [18]. Consequently, under FA @ 20 ppm and FA @ 10 ppm, cold incidence (23.96% and 22.29%) **(**Fig. 1) was likewise demonstrated to be higher. One possible explanation for plants' vulnerability to these treatments could be their inability to activate osmoregulation mechanisms during their active stage of development [19]. Again, a favorable link (Fig. 2, 3, 4) has been shown between fusaric acid treatment and the incidence of cold and wilt, and it might be due to the shorter duration of phenophases as depicted in the PCA biplot analysis, which showed a negative association with cold and wilt incidence.

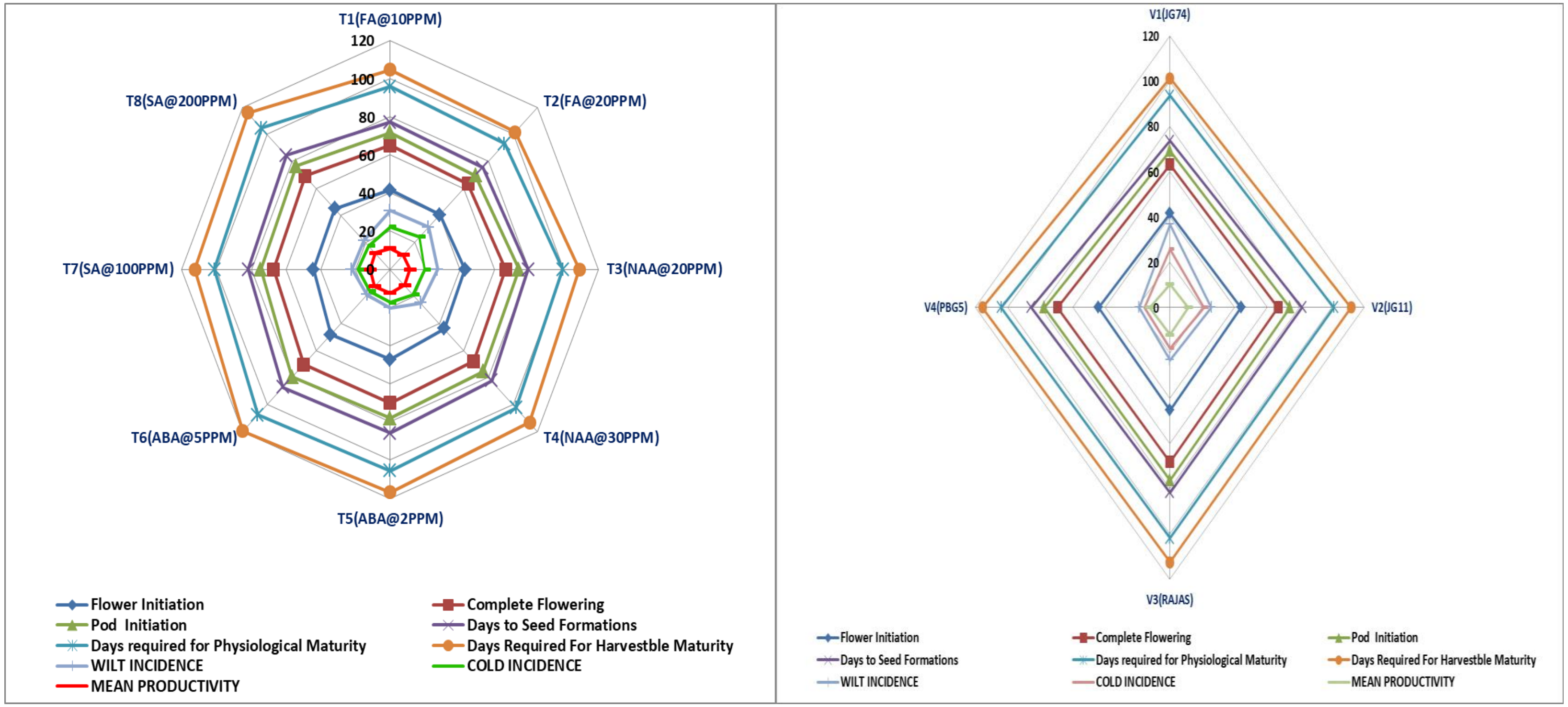

Fig. 1. Effects of different PGRs spray and varietal difference on phenophases, wilt & cold incidence and mean productivity of wilt and cold stressed chickpea

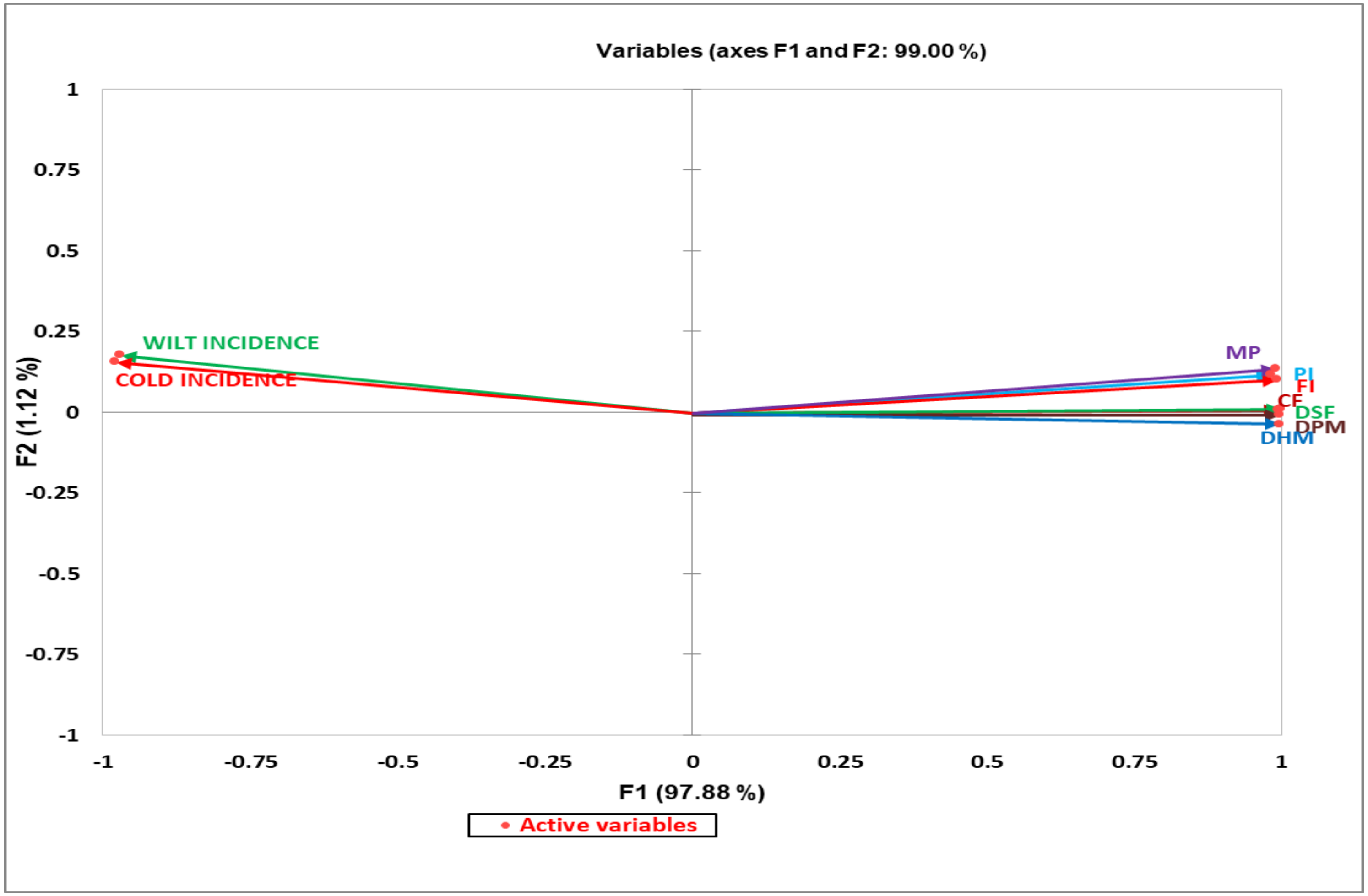

Fig. 2. Relationship study between traits under investigation where MP – mean productivity, PI – days required for pod initiation, FI - days required for flower initiation, CF - days required for complete flowering, DSF - days required for seed formation, DPM- days required for physiological maturity and DHM- days required for harvestable maturity

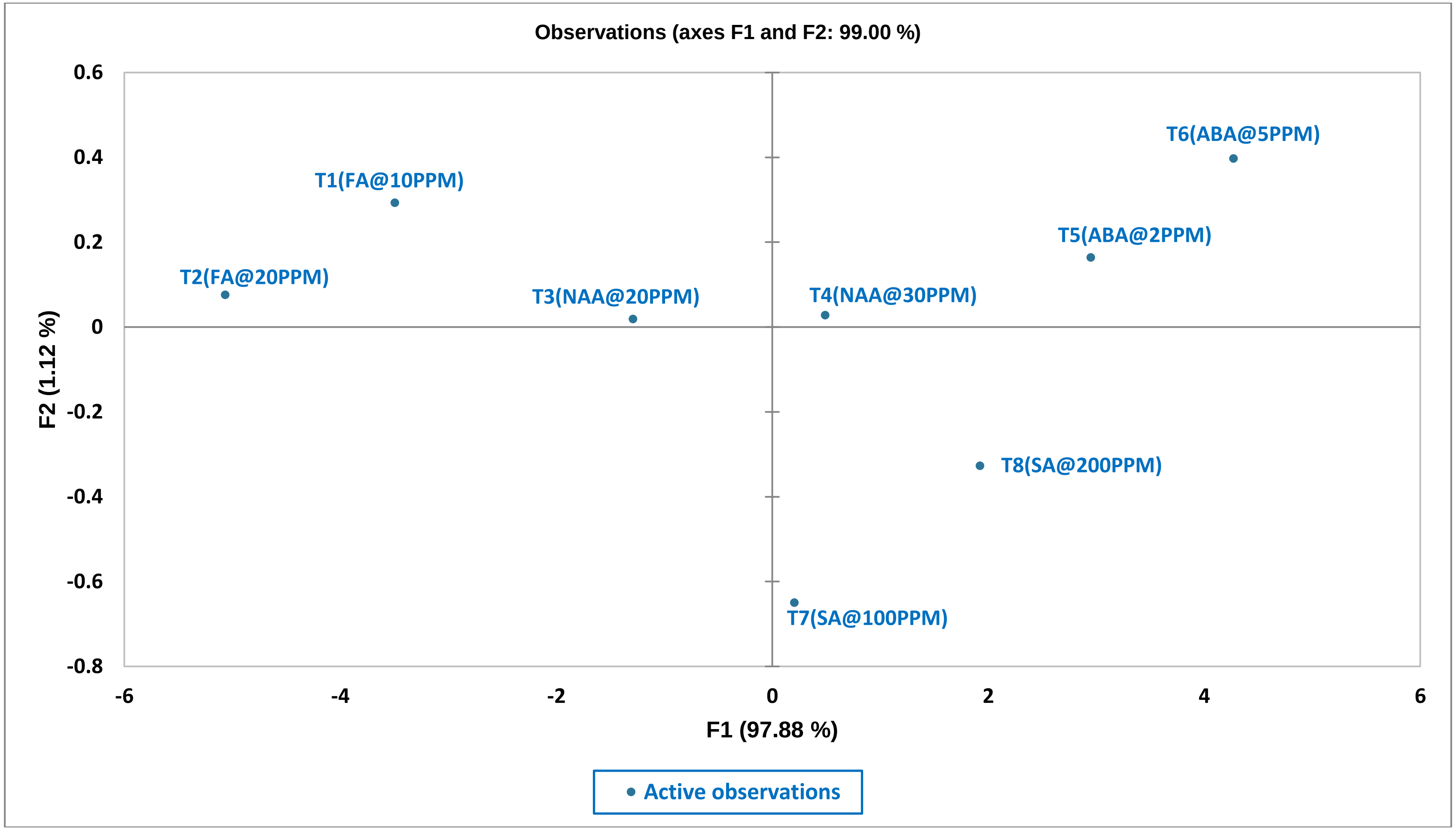

Fig. 3. Relationship study between different PGRs spray in wilt and cold stressed chickpea

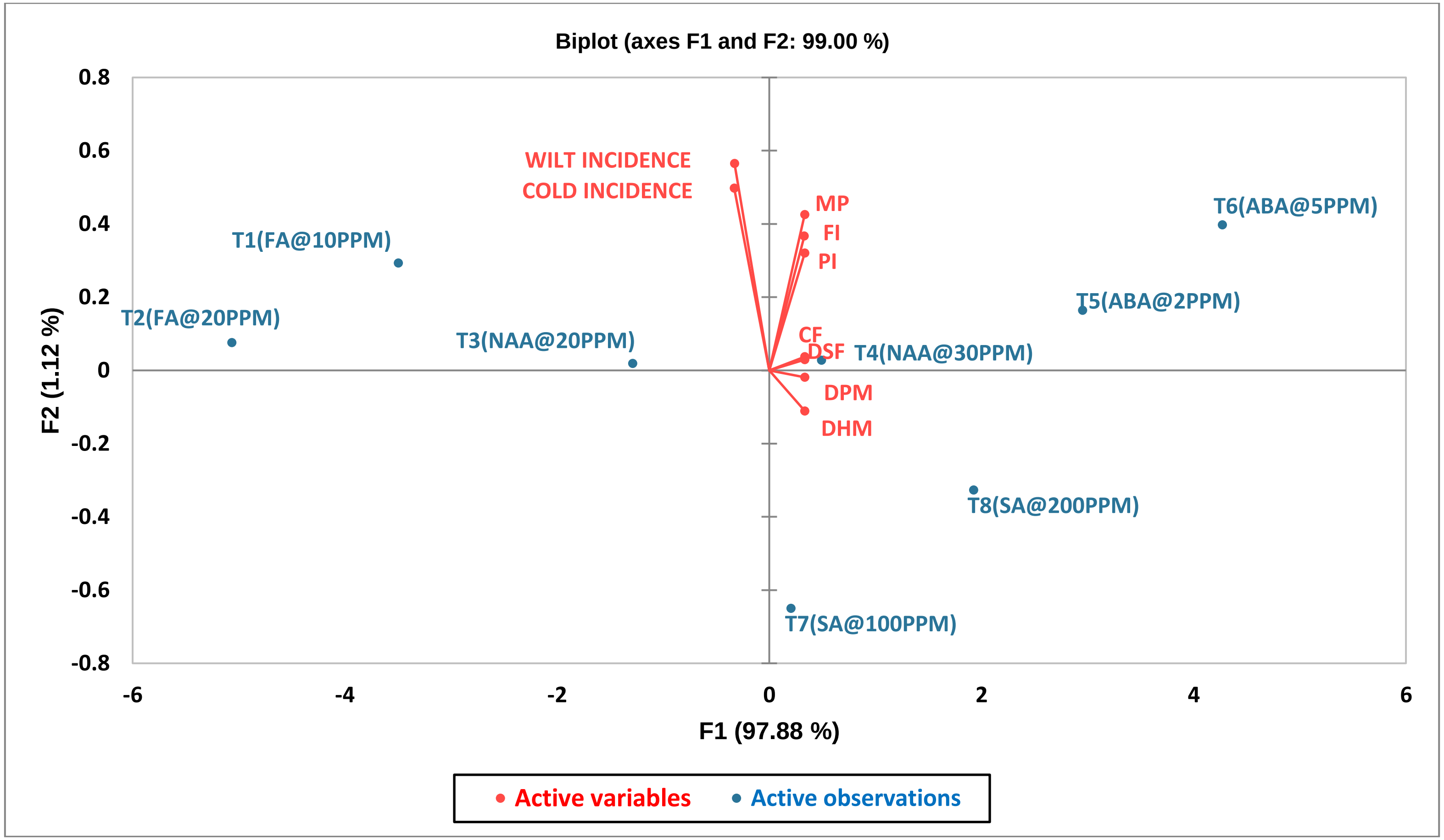

Fig. 4. Combined study of relationship between PGRs spray and there effect on traits under investigations in wilt and cold stressed chickpea

After artificial wilt inoculation, plants receiving ABA @ 5 and ABA @ 2 ppm were the least wilted (18.75 and 20.21%) **(**Fig. 1). It might be due to the fact that ABA is a major PGR responsible for activating defensive mechanisms in infected plants through upregulation of ABA-dependent transcription pathways [20] or enhancement of tolerance-responsive gene expression, both of which are important for providing crop plant tolerance against potential stress damage [21]. Here, we can observe the relationship between ABA treatment and phenophase delay, which allows the plant more time to express the genes, involved in the defensive system or activates its own defense mechanism, ultimately leading to tolerance and a lower wilt incidence in comparison to other PGRs that were used in our study.

It was also discovered **(**Fig. 1) that with both treatments, the percentages of cold occurrence were reduced, at 16.46% and 17.29%. This result could be explained by the fact that plants biosynthesize various types of small molecules known as stress proteins, or heat-shock proteins (Hsp), in response to wilt stress. These molecules may act as stress-mitigating agents by maintaining the water potential in cell sap and forming a barrier against membrane disruption and solute leakage, giving the plant the ability to cope with stress by protecting intracellular activity [22]. Based on earlier findings, it is now evident that delayed phenophases following ABA application at 5 ppm and 2 ppm are crucial in reducing the percentage of cold and wilt occurrences. The cultivar JG 74 (which is prone to wilt) had the highest incidence of wilt (36.67%), while the lowest (18.72%) was found in PBG 5.

Due to wilt pathogen disruptions in crop plant structure and functional activities, JG 74 also showed an increased incidence of cold (25.83%) **(**Fig. 1). All other cultivars utilized in the research had a higher cold incidence than PBG 5 (15.52%). Under simulated exposure to wilt and cold, the plant sprayed with ABA at 5 ppm and ABA at 2 ppm was able to sustain its stress tolerance response and displayed its maximum mean productivity (12.73 & 12.33 g plant-1). Similarly, the best mean productivity (12.50 g plant-1) was achieved by variety PBG 5. Since FA is a natural fungal toxin, substantial plant mortality happened when it was sprayed at 20 ppm and 10 ppm, which decreased plant productivity under these treatments. Genotype JG 74's mean production was likewise reduced (10.40 g plant-1) because of the obstacle imposed by stressful circumstances.

4. CONCLUSION

Plants sprayed with ABA @ 5 ppm and ABA @ 2 ppm effectively manage the damage caused by the wilt at an early stage and the cold at a later stage, thus providing tolerance to the crop to withstand unfavorable conditions and minimizing the yield loss due to the impact of wilt and cold. We discovered a strong and positive correlation between phenology and hormones by using PCA biplot analysis. Hormones that lengthen the life of crop plants may also help in lowering the probability of cold and wilt, same was found in the cases of ABA @ 5 ppm and ABA @ 2 ppm. Similarly, variety PBG 5 performed well under both (wilt and cold) adverse-induced conditions due to its longer life span.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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