

Original Research Article

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

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 against artificially inoculated *Fusarium oxysporum* (wilt pathogen) and cold exposure in four chickpea varieties. Additionally, the relationship between traits and PGRS application was looked at to evaluate their role toward 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 blossoming, 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.

Key point: Phenology, Abscisic Acid (ABA), Fusaric Acid (FA), Correlation, Stress Tolerance, Mean Productivity

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 the 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). By extending the duration of fruit and flower drop, 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 stress circumstances (8). Therefore present investigations are aimed to study the Relationship between Phenology, Stress Tolerance, and Mean Productivity in Cold Stressed and Wilted Chickpeas (*Cicer arietinum* L.) after application of different Synthetic PGRs.

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 Absciscic 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.

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

Phenological 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.

Wilt incidence (%): After applying a 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 (1986) (10) methodology was used to determine the percentage wilt incidence.

Cold incidence (%): It was calculated using the method proposed by Mayee and Datar (1986) (10), with appropriate modifications.

Mean productivity - Mean Productivity was calculated by using formula -

$$MP = (Y_{pi} + Y_{si}) / 2$$

Y_{si} and Y_{pi} are the mean grain yields of individual treatments under stressed and non-stressed conditions.

Statistical analysis - The data were statistically analyzed through completely randomized design given by Fisher (1955) and comparison of mean was performed on the basis of least significant difference test (LSD) according to method given by Gopinath *et al.*, (2021) (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.

Result and Discussions

From the pooled data obtained from year (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 (48.39 & 47.20 DAS), complete flower formation (70.40 & 69.95 DAS), pod initiation (79.86 DAS) and seed development (87.35 DAS) was taking place 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 its reproductive growth cycle are also attributed to longer vegetative growth durations (12). A PCA-based biplot analysis was used to attribute the relationship of plant modulators and traits that show stress tolerance and boost crop plant 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 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 by using PCA-biplot analysis's negative correlation was found between ABA @ 5 PPM, ABA @ 2 PPM with fusaric acid treatment @ 20 ppm and 10 ppm. This resulted in the earliest flowering (40.24 & 41.57 DAS) and complete flower formation (63.28 & 64.83 DAS) and also less the 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).

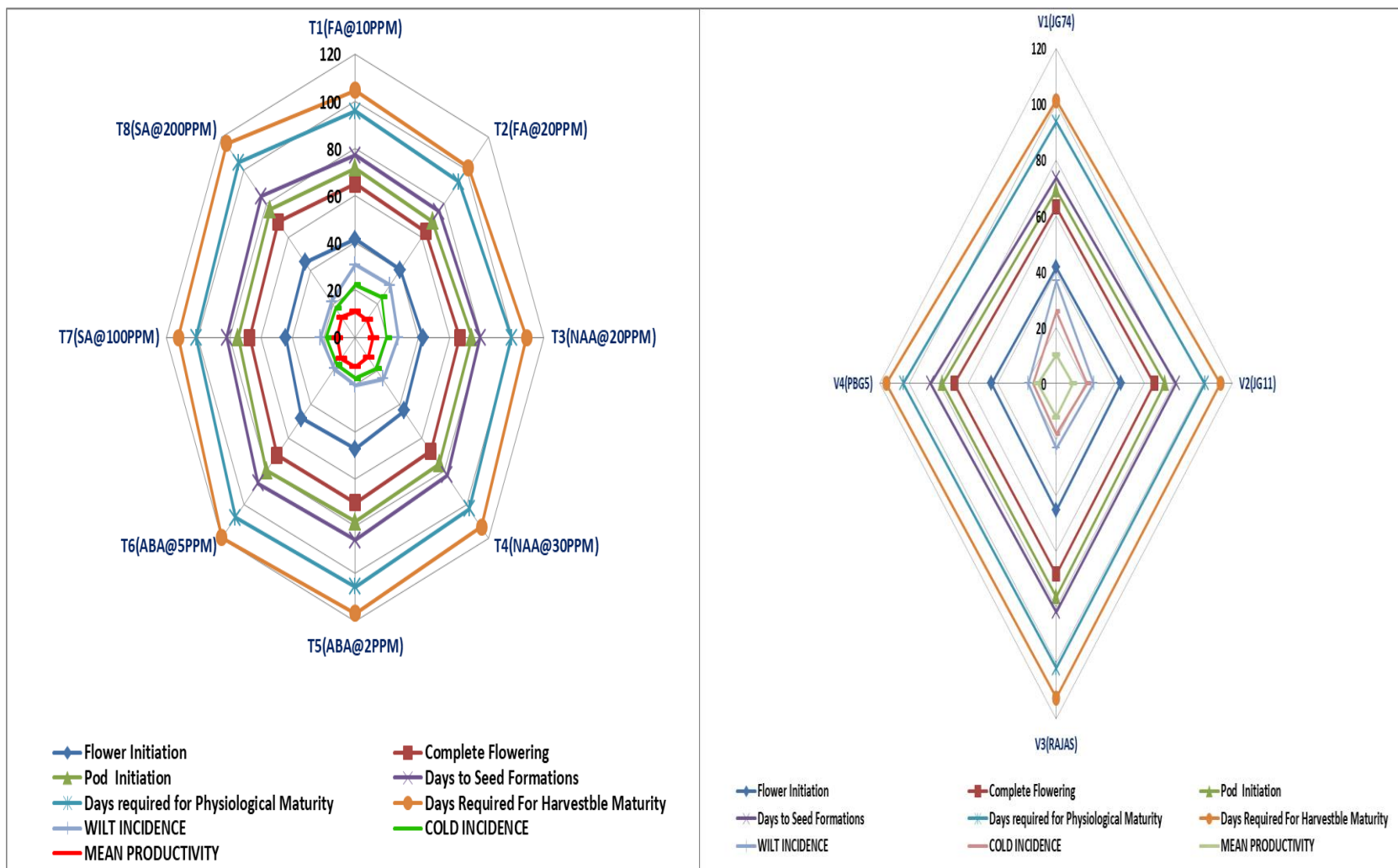


FIGURE1: Effects of different PGRs spray and Varietal difference on Phenophases, Wilt & Cold incidence and Mean Productivity of wilt and cold stressed chickpea.

Variety V4 (PBG5) 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 V₄ (PBG5) required more number of days for pod initiation (79.86 DAS) and seed formations (87.35 DAS) due to its normal growth period resulted from its tolerance capacity to nullifying adverse impact imposed by wilt and cold, whereas V₁ (JG74) have requirement of less number of days for pod initiation (69.20 DAS) and seed formations (73.61 DAS) might be due to changes in growth period imposed by wilt and cold. Similar findings was also observed by (14), who discover 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 find 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) increased as a result of treating the plants with ABA at 5 ppm and ABA at 2 ppm. Again both treatments had a positive correlation with both these traits and achieve higher value than that of other treatments. Whereas due to negative correlation of Fusaric acid treatment @ 20 ppm and @ 10 ppm with ABA at 5 ppm and ABA at 2 ppm, resulted in a shorter time to acquire physiological (92.90 & 95.54 DAS) and harvestable maturity (101.70 & 104.50 DAS) under of Fusaric acid treatment @ 20 ppm and @ 10 ppm. Our results are consistent with the findings of (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, 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 maximum days. Stress tolerance and susceptibility traits vary among genotypes, and this is reflected in the range in days needed for physiological maturity and harvestable maturity (16). For physiological development and harvestable maturity, genotype V4 (PBG5) needs the greatest number of days, while genotype V1 (JG 74), which is more sensitive, needs the fewest days.

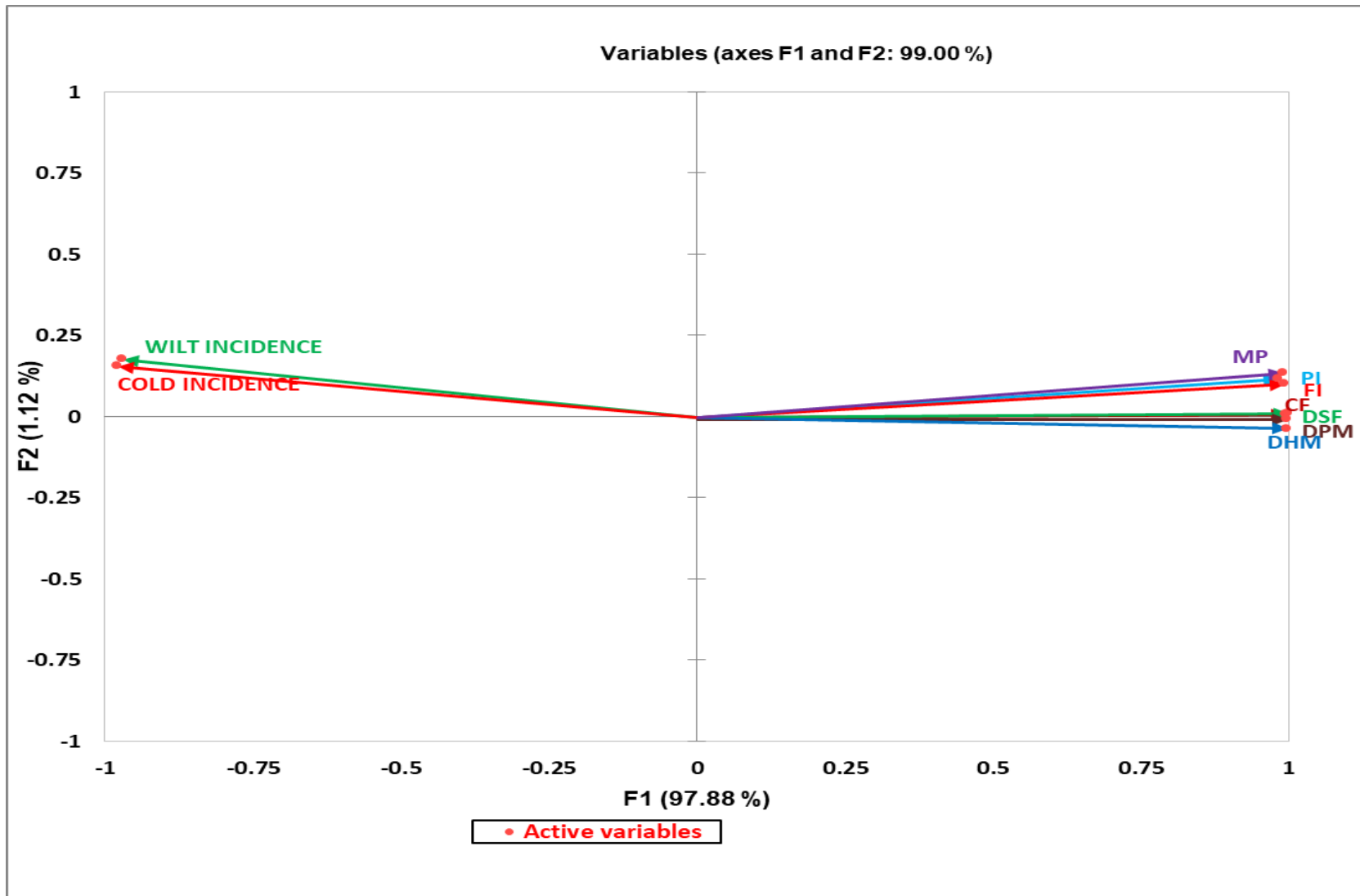


FIGURE 2: Relationship study between traits under investigation where MP – mean productivity, PI – days required for pod initiation, FI - days required for flower initiation, CF - days required for complete flowering , DSF - days required for seed formation, DPM- days required for physiological maturity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

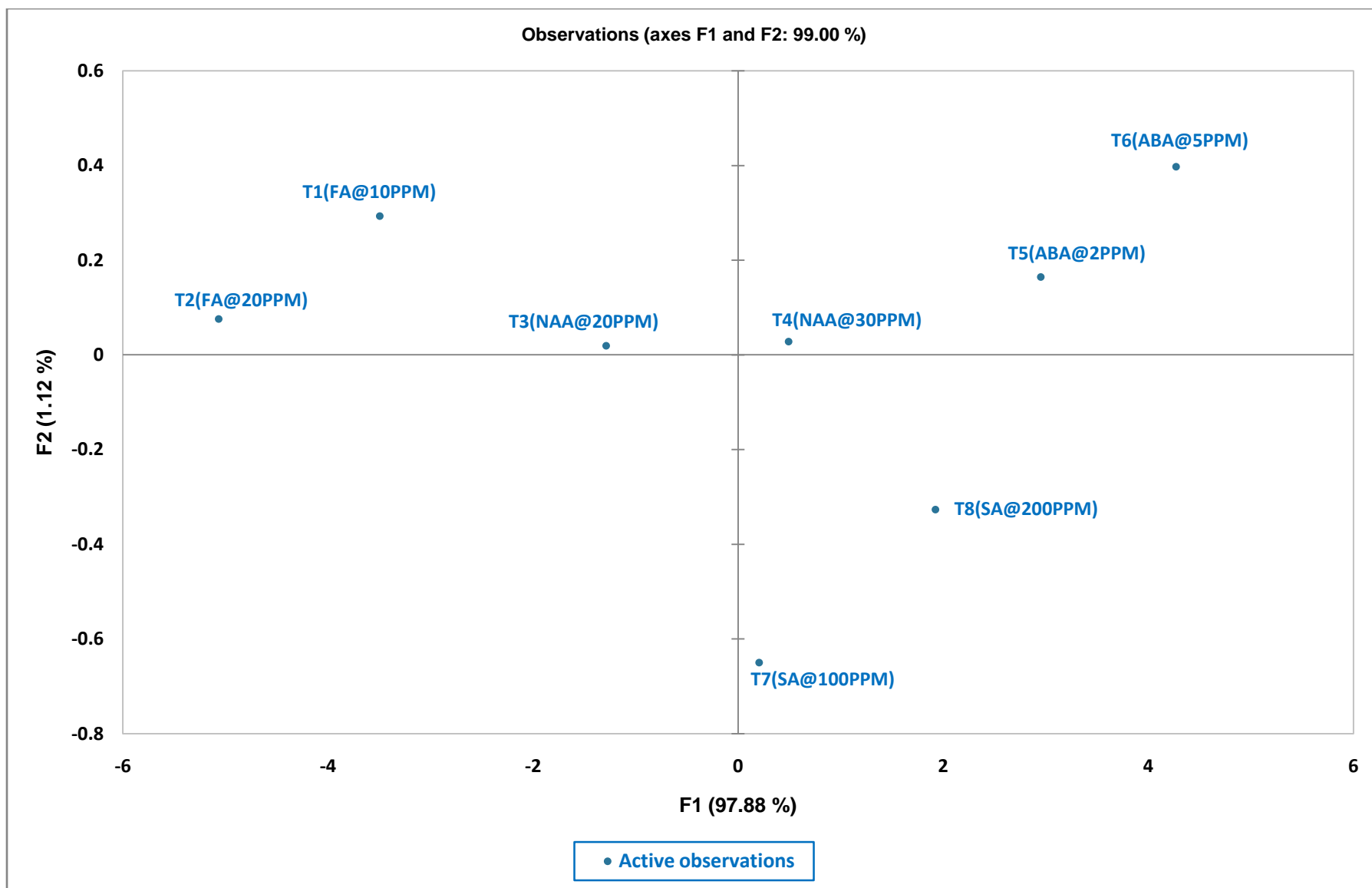


FIGURE 3: Relationship study between different PGRs sprayin wilt and cold stressed chickpea.

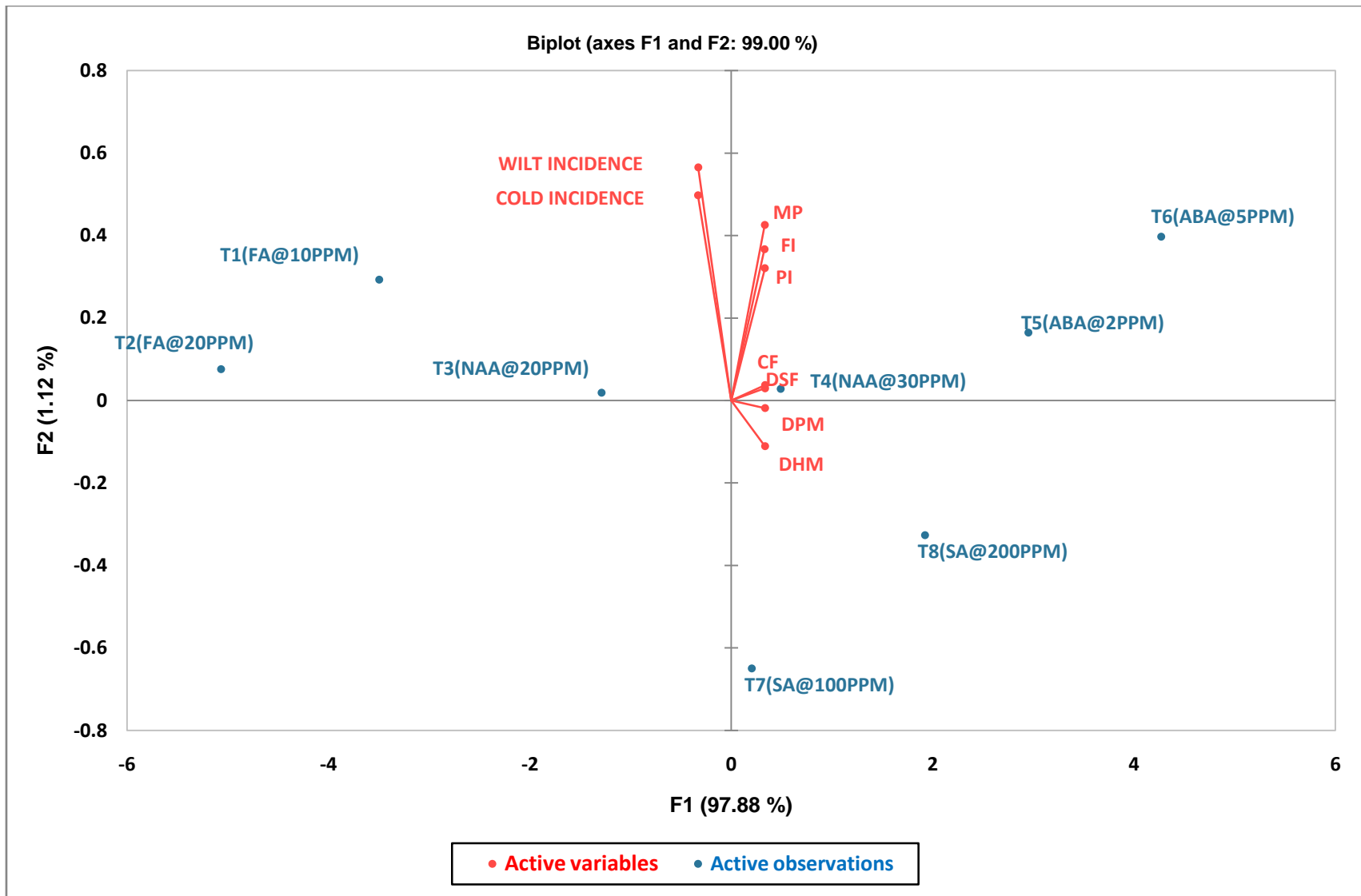


FIGURE 4: Combined study of relationship between PGRs spray and there effect on traits under investigationsin wilt and cold stressed chickpea.

Pooled data from both years (2020-2021 and 2021-2022) shown 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%) 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 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.

After artificial wilt inoculation, plants receiving ABA @ 5 and ABA @ 2 ppm were the least wilted (18.75 and 20.21 %). It might be due to ABA is a major PGR responsible for activating defensive mechanisms in infected plants through up regulation 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 have lower wilt incidence in comparison to other PGRs which was used in our study.

It was also discovered 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 occurrence. The cultivar JG 74 (which is prone to wilt) had the highest incidence of wilt (36.67%) 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%). 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 on by stressful circumstances.

Conclusion

Plant sprayed with ABA @ 5 ppm and ABA @ 2 ppm effectively manage the damaged caused by the wilt at early stage and cold at later therefor provided tolerance to crop to withstand under unfavorable conditions and minimize the yield loss due to impact of wilt and cold. We discovered a strong and positive correlation between phenology and hormone by using PCA biplot analysis. Hormone that lengthens the life of crop plants may also help in lowering the probability of cold and wilt, same was found in case of ABA @ 5 ppm and ABA @ 2 ppm. Similarly variety PBG 5 performed well under both (wilt and cold) adverse induced conditions due to higher the life span.

References

1. Anonymous 2019. ANNUAL REPORT, Department of agriculture cooperation and farmer's welfare Ministry of Agriculture & Farmers Welfare Government of India
2. Kaur R and Prasad K. 2021. Technological, processing and nutritional aspects of chickpea (*Cicer arietinum*). A review. Trends Food Sci. Technol. 109, 448–463.
3. Achari SR, Mann RC, Sharma M and Edwards J. 2023. Diagnosis of *Fusarium oxysporum* f. sp. *ciceris* causing *Fusarium* wilt of chickpea using loop-mediated isothermal amplification (LAMP) and conventional end-point PCR. Sci Rep. 13(1):2640.
4. Rani A, Kiran A, Sharma KD, Prasad PVV, Jha UC, Siddique KHM and Nayyar H. 2021. Cold Tolerance during the Reproductive Phase in Chickpea (*Cicer arietinum* L.) Is Associated with Superior Cold Acclimation Ability Involving Antioxidants and Cryoprotective Solutes in Anthers and Ovules. Antioxidants (Basel).10(11):1693.
5. Asha Kiran, Sanjeev Kumar, Harsh Nayyar and Kamal Dev Sharma. 2019. Low temperature-induced aberrations in male and female reproductive organ development cause flower abortion in chickpea.42 (7):2075-2089.
6. Banday ZZ and Nandi AK. 2015. Interconnection between flowering time control and activation of systemic acquired resistance. Front. Plant Sci. 6, 174.
7. Biasi R, Brunori E, Ferrara C and Salvati L. 2019 Assessing Impacts of Climate Change on Phenology and Quality Traits of *Vitis vinifera* L.: The Contribution of Local Knowledge. *Plants*, 8, 121.
8. Zhang Y, Xu J, Li R, Ge Y, Li Y and Li R. 2023 Plants' Response to Abiotic Stress: Mechanisms and Strategies. Int J Mol Sci.; 24(13):10915.
9. Thakur S, Tiwari G, Kumawat RK, Kumhare A, Singh S, Singh R. 2023. Synthetic PGR's Modify Phenology, Stress Tolerance and Mean Productivity in Wilt and Cold Stressed Chickpea (*Cicer arietinum* L.). International Journal of Environment and Climate Change. 13(11):1055-68.
10. Mayee CD and Datar VV 1986. Phytopathometry. Marathwada Agric. Univ. Parbhani Tech. Bull.-1: 66
11. Gopinath Pratheesh P , Parsad Rajender , Joseph Brigit and Adarsh VS. 2021. grapesAgri1: Collection of Shiny Apps for Data Analysis in Agriculture. Journal of Open Source Software. 6(63):34-37
12. Santiago Julián Kelly, María Gabriela Cano, Diego Darío Fanello, Eduardo Alberto Tambussi, Juan José Guiamet, 2021, Extended photoperiods after flowering increase the rate of dry matter production and nitrogen assimilation in mid maturing soybean cultivars, Field Crops Research, 265: 108104.
13. Singh VK, Singh HB, Upadhyay RS. 2017 Role of fusaric acid in the development of 'Fusarium wilt' symptoms in tomato: Physiological, biochemical and proteomic perspectives. Plant Physiol Biochem. 118:320-332.

14. Veeramani P and Sendhilvel V. 2020. Evaluation of Chickpea (*Cicer aritinum* L.) Varieties against Wilt Disease in North Eastern Hilly Zone of Tamil Nadu J Krishi Vigyan, 9 (1):114-117
15. Qi F and Zhang F 2020. Cell Cycle Regulation in the Plant Response to Stress. Front. Plant Sci. 10:1765. doi: 10.3389/fpls.2019.01765
16. Wabena Darkwa, Daniel Ambachew, Hussein Mohammed, Asrat Asfaw and Matthew W. Blair, 2016. Evaluation of common bean (*Phaseolus vulgaris* L.) genotypes for drought stress adaptation in Ethiopia, The Crop Journal, 4(5): 367-376,
17. Perincherry L, Lalak-Kańczugowska J and Stępień Ł. 2019 *Fusarium*-Produced Mycotoxins in Plant-Pathogen Interactions. Toxins (Basel). 11(11):664.
18. Seleiman MF, Al-Suhaibani N, Ali N, Akmal M, Alotaibi M, Refay Y, Dindaroglu T, Abdul-Wajid HH and Battaglia ML 2021. Drought Stress Impacts on Plants and Different Approaches to Alleviate Its Adverse Effects. Plants (Basel); 10(2):259.
19. Zhao S, Zhang Q, Liu M, Zhou H, Ma C and Wang P. 2021. Regulation of Plant Responses to Salt Stress. Int J Mol Sci. 22(9):4609.
20. Tischer SV, Wunschel C, Papacek M, Kleigrew K, Hofmann T and Christmann A. 2017. Combinatorial interaction network of abscisic acid receptors and coreceptors from *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. U. S. A. 114 (38):10280–1028
21. Sabagh EL, Hossain A, Islam A, Dinajpur MS, Iqbal B, and Aftab MA, 2021. Prospective role of plant growth regulators for tolerance to abiotic stresses, Springer Nature Switzerland AG 1–38.
22. Sun W, Van Montagu M and Verbruggen N. 2002. Small heat shock proteins and stress tolerance in plants. Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression, 1577(1)1-9.

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