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Evaluation of seasonal variation in relation to secondary metabolite and biomass production of *Andrographis paniculata*

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Abstract

The present study is aimed at investigating the effects of variation in secondary metabolites and biomass production of *Andrographis paniculata*to obtainbetter yield and market value. The seasonal dynamics of *A. paniculata* was carried out on Kharifand Rabi seasons in two successive years at 2007 and 2008. *A. paniculata*genotype was screened to assess the andrographolide accumulation and itsyield components on two harvesting dates corresponding to calendar months of June and November. The results revealed that last week of October ideal for obtaining maximum dry herbage (931.3 kg/ha) and total andrographolide yield (29.05 kg/ha), respectively. On the basis of relative estimates of total andrographolide is higher in February (3.12%) than September (1.85%).

Keywords: *Andrographis paniculata*, seasonal variation, andrographolide content, Biomass yield

1. Introduction

Secondary metabolite biosynthetic mechanism in plants produces a wide range of chemical compounds.They generally regarded as a nonessential process that produced as by-products or plant waste. However, there is evidence that secondary metabolites such as alkaloids, phenolics and terpenoids may provide defence against infection [1]. Recent year, these secondary metabolites are attractive targets for the development of new pharmaceutical drugs, herbicides, cosmetics and pesticides [2, 3, 4]. *Andrographis paniculata* is well known plant in Southeast Asia. The active components of *A. paniculata* are diterpene lactones found in the aerial parts of the plant. The clinical studies have shown that extracts of *A.paniculata* are biologically active and used in traditional remedy for treatment of infectious fever-causing diseases, colic pain, and loss of appetite, irregular stools anddiarrhea [5]. The main diterpene lactones that have been isolated mainly from aerial parts of *A. paniculata* is andrographolide a bitter colourless crystal has been reported to possess protective effect against urothelial toxicity [6] and liver disorders including treatment of hepatitis[7].

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A more detailed understanding of secondary metabolism in plants and variation in secondary metabolites within genotypes in different season will not only complement studies on primary metabolism and growth habit, but will also have practical implications for the production of particular secondary metabolites. *A. paniculata* was chosen for study because it has been used as a drug for multiple clinical treatments and itcontains the andrographolide. The recent finding of the multiple health promoting properties of andrographolide, there is a high demand of *A.paniculata*biomass for andrographolide extraction. Therefore, good packages and practices of the biomass production with high andrographolide content need to be optimized for gaining highest market value. In the present study we have investigating the effects of cropping season in secondary metabolites and biomass production of *A. paniculata.*

2. Materials and methods

2.1 Plant material, physical & environmental condition and biomass production

The present study was confined to AP10 and AP2 a well characterise genotypes of IGKV Raipur. The genotype AP10 collected from Indore, Madhya Pradesh at 22°43‘ 31.13“N, 75‘51‘ 56.00“E, 1810 ft. Mean Sea Level (MSL) and AP2 was collected from Kanker (Chhattisgarh) at 20°16‘ 05.78“N81°29‘ 52.31“E, 1597 ft.MSL. These genotypes were evaluated at morphological, molecular and biochemical level in the Department of Plant Molecular Biology & Biotechnology, Indira Gandhi KrishiViswavidalya, Raipur, India [8] Based on the morphological, biochemical and molecular characterization of all genotypes, AP10 and AP2 were selected for the present study. The both genotypeswere used to assess the seasonal variation in biomass production and accumulation of andrographolide content at 21°14‘ 34.09“ N and 81°38‘ 19.35“ E(IGKV, Raipur) at an altitude of 981 ft.MSL.The average annual rainfall at this location is about 1200 mm, which normally occurs during late July to mid-September. The temperature range during summer is 29°C - 45°C and winter 10°C - 27°C. The experiment was conducted consecutively two year for confirmation of accuracy of result.

The genotypes were grown in sandy loam soil, with the bed size 5ft. x 5ft. with row to row spacing 45cmand plant to plant spacing of 30 cm under randomized block design. At the rate of 10 quintal/hectare well rotten FYM was used at the time of field preparation. No chemical fertilizer and plant protection measures were taken during the experiment. Although three hand weeding was done at 15 days interval after showing.Seed showing was done in the last week of June and first week of November. All cultural practices and data recordings were the same for both the seasonsand years of evaluation. Data were recorded on various morph agronomic traits such as plant height, number of branches, number of leaf, leaf length, leaf width, dry weight of plant for asses the biomass production.

2.2 Isolation and estimation of andrographolide

The HPLC used for the estimation of andrographolide accumulation. The young leaves of *A. paniculata*genotypes were collected in the month of September and February during Kharif and Rabi seasons respectively. The collected leaveswere dried in hot air oven (45°C) followed by grinding. The fine leaves powder samples used for extracting and quantifying the andrographolide. HPLC condition was optimized using a ODS C-18 column, mobile phase acetonitril :methanol : water (65 : 20 : 15), flow rate 1 ml per min, column pressure 88.7 bar and total run time of 10 min at 223 nm. The standard andrographolide (Sigma, USA) was usedin different concentrations (22mg, 11mg,

and 5.5mg) to calibrate reference curvefor quantification of andrographolide. The 20 µl of each concentration wasinjected into the HPLC. The reference pick obtained at 2.690 min retention time and the peak area corresponded to andrographolide amount in respect to standard solutionconcentration. For quantification of sample andrographolide, two grams powder sample of dried leaves were dissolved in HPLC solvent at the rate of 1000 factor and 20 µl of each were injected. The quantification was done in three replicates. The retention time and peak area were recorded for calculating the total andrographolide content in each genotypes.

2.3 Statistical analysis

Analysis of variance was performed to test the significance of differences between means obtained among the treatments in each experiment at the 5% level of significance ($p<0.05$).

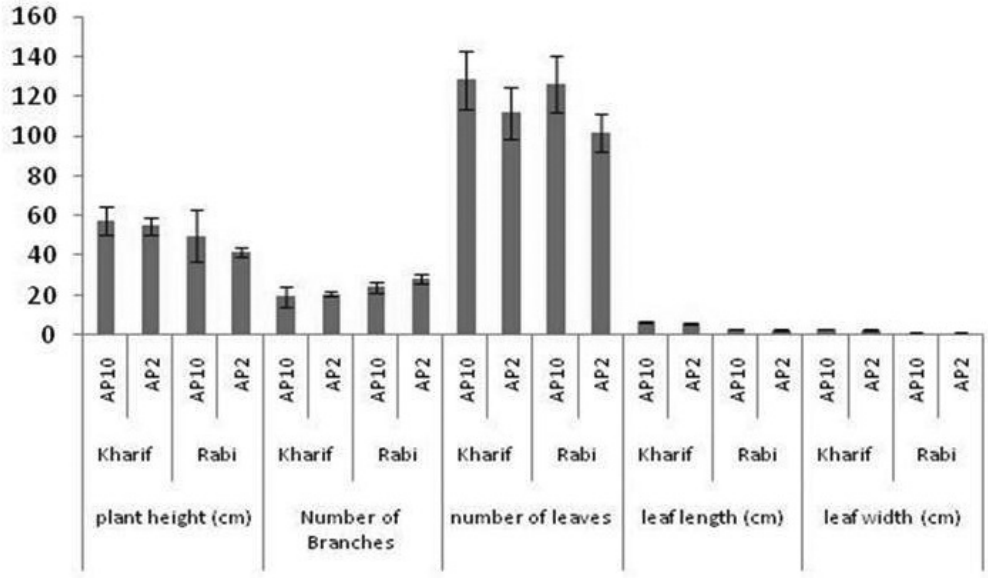

Fig. 1. Average estimation of yield related component during 2001 and 2008 Kharif and Rabi

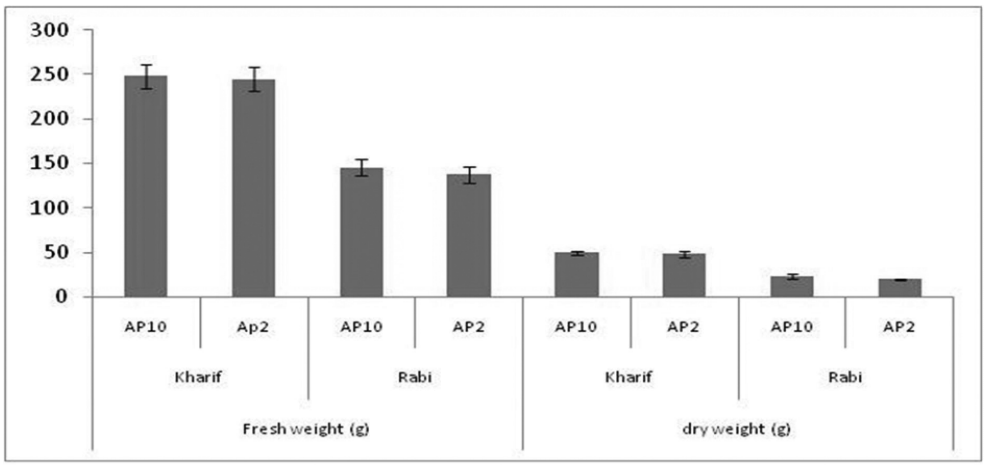

Fig. 2. Average fresh and dry weight of *A. paniculata* herb during 2007 and 2008

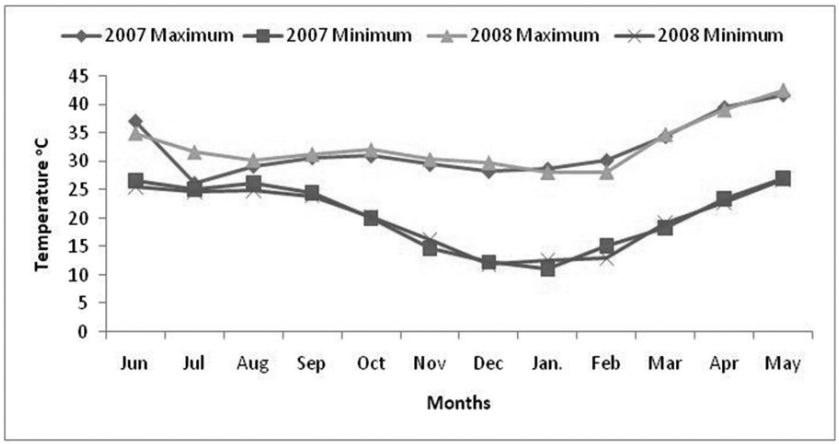

Fig 3: Average maximum and minimum temperature profiles during 2007 and 2008

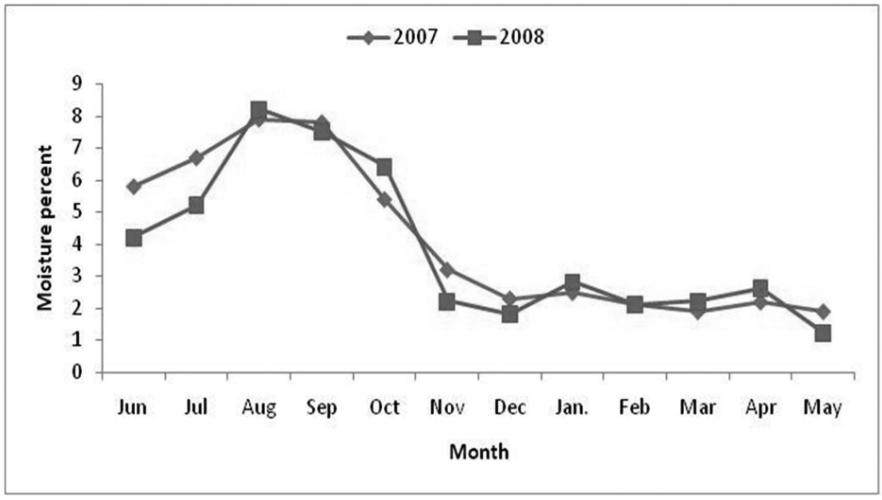

Fig 4: Average soil moisture content during 2007 and 2008

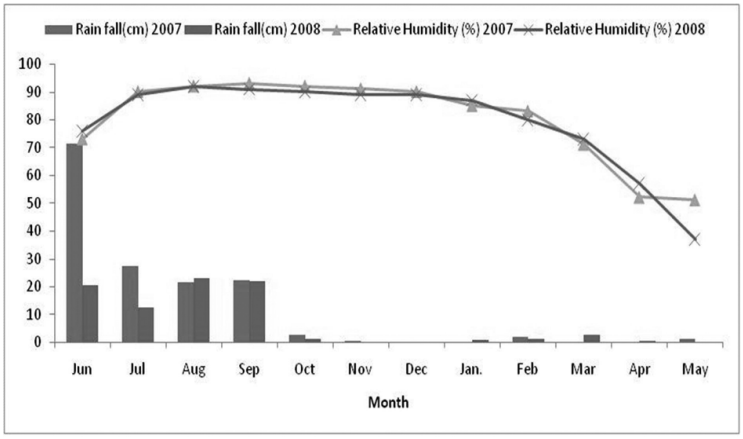

Fig 5: Other meteorological data during 2007 and 2008

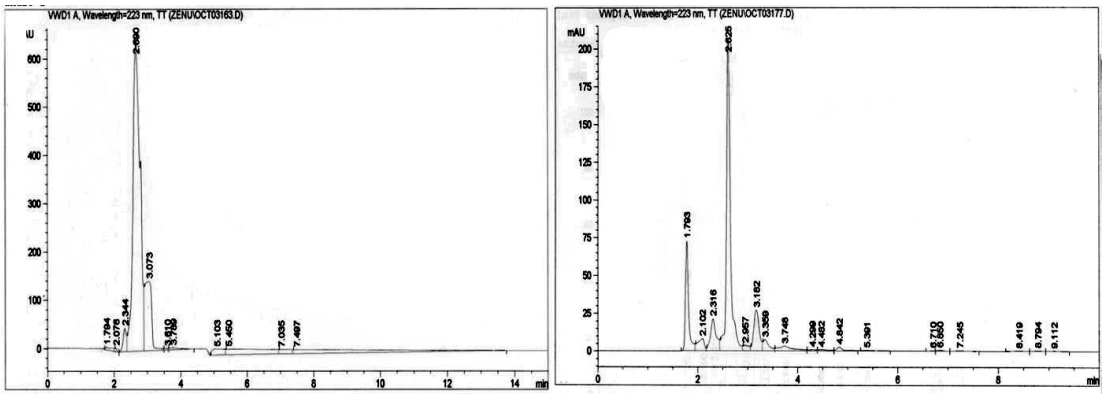

Fig 6:HPLC profiles for quantification of sample andrographolide

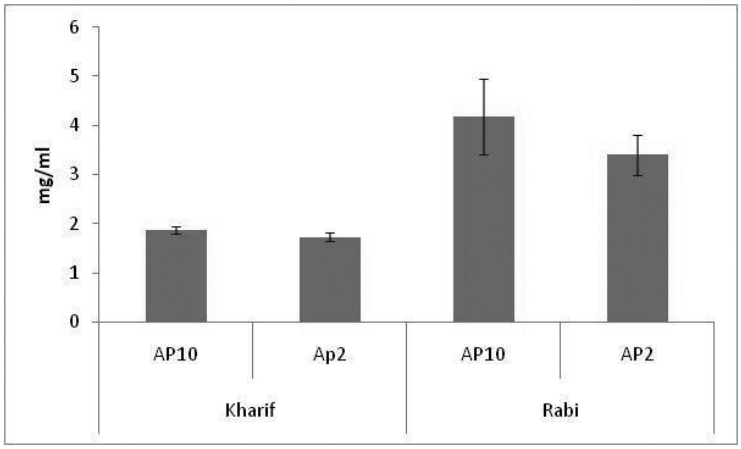

Fig 7: Comparison of average andrographolide accumulation of A. paniculata Herb during 2007 and 2008

3. Results and discussion

3.1 Seasonal variation and biomass production attributes

The assessment of biomass production carried out in two consecutive growing seasons 2007 and 2008. The different morphological traits was analyzing for evaluating seasonal variation. The mean value of morphological traits of the both years was cited in the manuscript because there was no significant difference recorded in two successive years. However, the morphological characters were shown significant variation between the seasons at 0.05 level of probability. Yield performance of the plants were estimated by measuring plant height, number of branches, leaf length, leaf width, fresh weight and dry weight of *A. paniculata*. The cropping season has considerable effete on growth parameter. The average estimation of yield related component during 2007 and 2008 Kharif and Rabi season has shown in fig 1 and fig 2. The results are as:

3.2 Plant height (cm)

Thedata regarding plant height (fig.1) was shown significant variation in both varieties under studied in different cropping seasons. Maximum plant height obtained during the Kharif (57.6±7.05 in AP10 and 54.6±4.02 in Ap2) than Rabi season. The AP10 attained 7.6% more plant height during Kharif than Rabi season where

AP2 attained 13.5% more plant height. Plant height one of the most important morphological character that significantly contribute the production of *A. paniculata*.

3.3 Number of branches

The cropping seasons also affect the number of branches. The results showed that more number of branches found during Rabi season than Karif seasons (fig1). The number of branches recorded 24.2±2.6 (AP10) and 28.4±2.3 (AP2)during Rabi season whereas 19.4±2.1 (AP10)and 20.6±1.14 (AP2)during Kharif seasons.

3.4 Number of leaves

The number of leaves directly related with the market value of *A.paniculata* because leaves have high amount of andrographolide and other lactones that have great medicinal properties and it is directly sale in the market in dry form. Seasons also affect the number of leaves significantly in both the genotypes (fig 1). The maximum number of leaves recorded during the Karif season it was 128.2±14.4 in AP10 and 111.8±12.7 in AP2. The 3.5% and 4.5% more number of leaves recorded in AP10 and AP2 respectively during the Karif seasons.

3.5 Leaf length and leaf width

Theleaf length and leaf width of *A. Paniculata* were exhibited significant variation in respect to the cropping seasons (fig1). The leaf length recorded 6.98±0.3cm in Karif season and 2.94±0.08 during Rabi in AP10, whereas the AP2 have 5.94±0.65 and 2.68±0.24cm during the Khari and Rabi seasons respectively. The leaf width recorded 2.96±0.15, 1.12±0.14 in AP10 and 2.64±0.26, 1.08±0.16 in AP2 during the Karif and Rabi season respectively.

4. Fresh weight & dry weight

The seasonalso significantly affects the fresh and dry weight of herb (fig 2). The fresh weight of herb was enhanced 18.95% and 28.17% in AP10 and AP2 respectively during Karif season. The fresh weight of AP10 was recorded 248.4±13.07g during Karif and 145.6±8.86g during Rabi. In the other hand the fresh weight of AP2 was recorded 245±12.3 and 137.3±8.5 during Karif and Rabi respectively. In case of dry weight AP10, AP2 was enhanced by 36.79% in and 42.08% during the Karif seasons respectively. The production of AP10 dry biomass weight was recorded 50±2.23g and 23.11±2.6g and AP2 produced 47.8±3.2 and 19.52±0.79 during the Karif and Rabi seasons respectively. Specific adaptability of genotypes explains the phenomenon of a genotype performing well in an environment [9]. In most of the crop species, vegetative growth and floral initiation is accelerated by high temperature. The environmental variations have strong influence on the crop phenology in addition to other traits [10]. The environmental factors also been intensively recorded during the crop seasons. The total annual rainfall during 2007 and 2008 was 1507mm and 840 mm respectively compared to long-term average of 1200 mm. Optimal growing season rainfall in kharif(last June to mid-November) during 2007 was 1467 mm and 2008 was 790mm respectively and optimal rainfall in Rabi (mid November to mid April) during 2007 was 35.5mm and 2008 was 49.2mm. In 2008, rainfall was at least 28.41% less than long-term average. The absolute maximum and minimum temperatures was 32.28°C and 20.3°C respectively during 2007, where as in 2008 it is 32.68°C and 20.1°C in later months due to mild rain.Overall, the metrological data was consistence in both the year. The other metrological data of both the years shows in fig 5. Climate and weather are the two major limiting factors in crop production even in secondary metabolite production that affect the price trends of agricultural produce. Therefore,itis always considered in the matter

of the time of sowing, transplanting, scheduling of irrigation, timing of fertilizer application, using of pesticides etc (Fig. 3). The availability of soil moisture during cropping seasons contribute significant role in biomass production and accumulation of secondary metabolite. Soil moisture contents of 2007 and 2008 not showed significant difference among the monthsalthough minor variation was present (Fig.4). In 2008, soil moisture was comparatively better in 2007 in later months due to mild rain.Overall,the metrological data was consistence in both the year. The other metrological data of both the years shown in (fig 5) Climate and weather are the two major limiting factors in crop production even in secondary metabolite production that affect the price trends of agricultural produce. Therefore, itis always considered in the matter of the time of sowing, transplanting,scheduling of irrigation,timing of fertilizer applocation,using of pesticides etc.

4.1 Seasonal variation in andrographolide accumulation

The HPLC used for estimation of seasonal accumulationof andrographolide [8]. It is simple fast,sensitive and automated technique with higher degree of resolution. The HPLC profile of the slandered andrographolide was prepared and shown in fig 6. In standard pick, andrographolide had a stable and high content, and could be separated completely from the other peaks. Accordingly reference peak was selected. The presence of andrographolide in *A. paniculata* samples were identified and estimated by comparison of their retention time with the standard chromatogram (fig.6). The area under peak correspond to amount of andrographolide in the sample was confirmed by comparing the spectrum obtained by photodiode array detector, which was completely in agreement with the standard. The andrographolide isolation and quantification were performedduring the month of September and February in the year of 2007 and 2008. There was significant seasonal variation recorded in andrographolide accumulation in leaves. The andrographolide accumulation declined during the Kharifseason at a rate of 2.92±0.07mg i.e., 43.84% in AP10 and 2.01±0.04 i.e. 36.21% The AP2 genotype (Fig. 7), As a result, the magnitude ofthe increment inandrographolide accumulation was nearly fourfold greaterin Rabi season than in Kharifseasons. The highest andrographolide accumulation (4.181±0.04 mg in AP10 and 3.4±0.4 mg in AP2) was recorded in Rabi seasons. The study suggested that the andrographolide content in the leaves of AP varied according to the seasonal factors Therefore, rational utilization of the AP plant samples or plant extracts for gaining maximum marketable value by assessing the variation in amount of andrographolide by considering environmental factor.Several factors such as changes in seasonal patterns, weather events, temperature changes, biotic and abiotic stresses may affect the production of biomass and secondary metabolites in plants [11, 12, 13]. The similar study on five oat cultivars located in different parts of Norway was shown considerable seasonal differences in deoxynivalenol (DON)content.The highest mean DON concentration was recordedin 1988 and 1989 because these years were characterized by heavy rainfall. The lowest DON concentration was recorded in 1987 and 1990 due to drought weather [14]. The study revealed that the some extent of stress either it is biotic or abiotic enhanced the secondary metabolite production for development of defense response in plant. The azadirachtincontent of *Azadirachtaindica*also varied from 200 to16,000 ppm (µg/g of the seed kernel) due to season and geographical condition. The highest azadirachtincontentwas recordedin the southern part of India [15]. The results of the present

study shown that accumulation of andrographolide and biomasswas affected by season and the genotypes.

Production of secondary metabolite is governed by both genetic and environmental factors [16]. Rainfall and temperature are the two critical factors affecting the production of secondary metabolites. Therefore, the effect of season on andrographolide content was studied. The study proved that the seasons affect morphological, physiological, quantitative and qualitative traits in *A. paniculata*. Significant differences were observed in andrographolide content of samples collected from Kharif and Rabi season in two successive years. It is important to mention here that less soil moisture with mild winter type ofclimate favors the andrographolide accumulation. Thus,moderate stress climatic conditions were found to favorable for andrographolide biosynthesis.

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