MATERIALS AND METHODS

The proposed investigation was carried out on morphological, physiological, biochemical and seed yield in *Panicum maximum* Jacq. as a response of foliar application of Naphthalene acetic acid (NAA), Diammonium Phosphate (DAP) and Muriate of potash (K₂O). The details of experimental sites, climatic conditions, choice of material and the methods applied have been described below.

**Experimental site**

The experiment was conducted in experimental field of Seed Technology Division at Central Research Farm of Indian Grassland and Fodder Research Institute, Jhansi (U.P.) India (at the latitude 25.27° N, longitude 75.35° E and an altitude of 275 meters above sea level) during the years 1997 - 98 and 1998 - 99. The soil of the experimental site was black and loams. The soil contained nitrogen and organic carbon (0.34 %), available phosphorous (23 kg P/ha), potash (315 kg K₂O /ha) and nearly neutral in reaction.

**Climatic conditions**

The climatic condition during the period of experimentation (1997-98 and 1998-99) is graphically presented in the figure 1. In 1997-98 the average maximum
temperature was recorded from 21.7 to 42.9 °C and the average minimum temperature was from 6.2 °C to 28.1 °C. During 1998-99 the average maximum temperature was from 21.9 – 42 °C and average minimum temperature ranged from 6.5 – 27.2 °C. The total rainfall was 986.4 mm in 1997, 905.6 mm in 1998 and 1118.2 mm in 1999. There was no much difference in the relative humidity in both the years. The highest value of evaporation was recorded in April-May and minimum values in the month of Dec-Jan. The bright sunshine hours/day was recorded maximum in the month of May that is about 10.2 hours/day during both the years.

Choice of material

*Panicum maximum* Jacq. commonly known as Ginni ghas in Hindi is a native of Tropical Africa and now it is cultivated in other tropical countries such as Uganda, Kenya, Jamaica, West Indies, Trinidad, Brazil, Australia, Malaysia, Srilanka, South East Asia and Philippines. In India it can be easily grown in areas with varied conditions of soil and climate particularly in tropical areas of Tamil Nadu, Kerla, Maharashtra, Andhra Pradesh and North India.

In *Panicum maximum* there are two different adaptive natures of cultivars, one (Panics) is suitable for medium rainfall of subtropics and other (Guineas) in high rainfall of humid tropic. This grass species is a tufted perennial, often with a shortly creeping rhizome variable 60 - 200 cm high, leaf blades upto 35 mm wide tapering to fine point, panicle 12 – 40 cm long, open spikelet 3 - 3.5 mm long, obtuse, mostly purple, glumes unequal, the lower one being one third to one fourth as long as the spikelet, lower floret usual male, upper floret (seed) distinctly transversely wrinkled.

Warm and humid condition with partial cloudiness and light showers of rain favor the rapid growth of this grass. The minimum temperatures should remain above 15 °C but the maximum temperatures beyond 38 °C do not favour this grass. As a pasture grasses, it requires a minimum rainfall of about 600-1000 mm and a relative humidity of 70%. It can tolerate rainfall upto 1200-1500 mm. The grass grows well from sea level to an elevation of 2000 meters. Hot and dry desiccating wind in the summer and cold condition in the winter as prevail in the north, are not congenial for its growth.
Looking wide adaptability and economic use the *Panicum maximum* was selected for this study. The major objective of the investigation was to study the influence of foliar application of growth hormone (NAA) and nutrients (DAP and muriate of potash) on morpho-physiological characters; biochemical attributes, assimilatory functions, seed yield and seed yield contributing characters of *Panicum maximum*.

The experiment was laid out in plot size 4 x 4m with three replication in RBD. Fourteen treatments were given in single and in combination of growth hormone and nutrients, which are as follows:

**Treatments:**

| T1   | : | Control               |
| T2   | : | 10 ppm NAA            |
| T3   | : | 50 ppm NAA            |
| T4   | : | 100 ppm NAA           |
| T5   | : | 2 kg DAP/ha           |
| T6   | : | 4 kg DAP/ha           |
| T7   | : | 6 kg DAP/ha           |
| T8   | : | 2 kg K/ha (Muriate of potash) |
| T9   | : | 4 kg K/ha             |
| T10  | : | 6 kg K/ha             |
| T11  | : | 2 kg/ha DAP + 2 kg K (Muriate of potash) |
| T12  | : | 10 ppm NAA + 2 kg DAP/ha |
| T13  | : | 10 ppm NAA + 2 kg K/ha |
| T14  | : | 10 ppm NAA + 2 kg K/ha + 2 kg DAP |

The treatments were applied foliarly at vegetative stage (45 Days After Germination, DAG) and flower initiation stage (65 DAG) of the plant. In the control plot the crop was sprayed with water.
Crop establishment

For crop establishment seedlings were raised in the nursery during June 1997. The healthy eight months old seeds were sown in nursery beds at 0.5 to 1.0 cm. soil depth on 15th June. One month old seedlings were transplanted in well prepared field in plots (4 x 4m) with three replications in Randomized Block Design. Before sowing the soil samples were taken for the analysis of nutrient status present in the soil. The plots were fertilized with basal dose of 60 kg N/ha and 40 kg P₂O₅/hacter. Potash was not given, since the soil was rich in potassium content. After transplanting and establishment the weeding and inter culturing was done at proper time during the growth of crop. The treatments of different concentration of NAA, DAP and K₂O were foliarly applied at vegetative stage and flower initiation stage of crop growth. All the morphological, physiological and biochemical characters were recorded at 50% flowering stage. Seed yield contributing characters and seed yield were recorded at the time of seed maturation. After seed harvesting the biomass was harvested and plots left for the experimentations for second year, as the crop is perennial in nature.

During second year the plants regenerated just after first shower of monsoon in the last week of June. After regeneration inter culture, weeding and other agronomical practices were carried out for better crop growth. 30 kg N/ha was applied as basal dose. The foliar treatments of NAA, DAP and K were given after 45 days of regeneration and also at flower initiation stage.

Recording of observations

Morpho-physiological parameters

The morphological parameters of plant such as plant height, number of tillers number of leaves, leaf area, root length, fresh and dry weight was recorded at 50% flowering stage. For taking the fresh weight the plants were washed properly in running water and blotted to remove extra surface water before weighting.

The leaf area of fresh leaf was measured by using automatic portable leaf area meter (Model LI-3000, LICOR USA), before weighing. The samples of each plant part
were dried in an electric oven at 80 °C for 48 hours and then the dry weight was recorded.

**Specific leaf weight (SLW)**

The ratio of leaf dry weight (LW) to leaf area (LA) was used for calculation of SLW

\[
\text{SLW} = \frac{\text{Leaf weight (LW)}}{\text{Leaf area (LA)}} \quad \text{(mg cm}^{-2}\text{)}
\]

**Assimilatory characters**

The physiological observations such as micro-environmental parameters, rate of photosynthesis (PN), stomatal conductance (CS), intercellular CO₂ concentration (CINT) and transpiration rate (TR) were recorded in the leaves using the LI - 6250 Portable photosynthesis system (LI-COR, USA). Photosynthetically active radiation (PAR), air temperature (AT), and relative humidity (RH) were also measured during the recording of physiological observations. All the measurements were made at ambient CO₂ between 11.00 and 12.00 hour on a clear sky day. The ratio of PN/TR, PN/CINT was also calculated. Leaf transpiration was also measured at the time of recording these physiological observations. All these observations were made in fully expanded healthy second and third leaves of the plant.

**Chlorophyll accumulation**

Photosynthetic pigments (Chlorophyll) in fresh leaves were estimated by the method of Duxbury and Yentish (1956). One gram of fresh leaves was homogenized in 80% acetone and supernatant was filtered through funnel using filter paper in 50 ml volumetric flask and made volume with acetone. A suitable amount (one ml) of aliquot was taken in 25 ml of volumetric flask and volume was made upto 25 ml with acetone. Concentration was measured by measuring the absorbance of solution using UV-spectrophotometer at 660 μm and 640 μm wavelengths for chlorophyll a (Chl a) and chlorophyll b (Chl b) respectively. Finally the fraction of chlorophyll were calculated
from the absorbance value using following equations given in A.O.A.C. (1970) and expressed in mg chl /gm of the fresh weight of leaves.

\[
\begin{align*}
\text{Chl a} &= 9.93 \log 10 \frac{I_0}{I(660 \text{ m}\mu)} - 0.777 \log 10 \frac{I_0}{I(640 \text{ m}\mu)} \\
\text{Chl b} &= 17.3 \log 10 \frac{I_0}{I(640 \text{ m}\mu)} - 2.8 \log 10 \frac{I_0}{I(660 \text{ m}\mu)}
\end{align*}
\]

Total Chlorophyll = Chl a + Chl b

**Sugar contents**

Sugar content in leaves or plant was determined by Anthrone method. 100 mg of fine powdered dry sample was placed in 15 ml centrifuge tube and 10 ml of 80% ethanol was added. The tube was covered with glass ball and then kept in a water bath at 80 °C for 30 minutes, then centrifuged and supernatant was decanted in 50 ml beaker. This extraction was repeated at least for three times. Finally the alcohol is evaporated from extract on water bath at 80 – 85 °C and volume was made in a 25 ml volumetric flask with distilled water. Then 5 ml aliquot was taken into test tube and kept in ice bath. 10 ml of anthrone reagent was added slowly in each tube from side of test tube and stirred slowly with a glass rod and then placed on boiling water bath for 7 to 8 minutes and cooled immediately in ice bath. After cooling the absorbance was measured at 630 m\(\mu\) and the sugar percentage in the sample was calculated from the standard curve prepared from glucose.
NUTRIENT CONTENTS

Nitrogen

Total nitrogen in plants was estimated by micro Kjeldahl method (A.O.A.C., 1960). 100 mg oven dried sample was taken in a dry micro Kjeldahl flask containing 5 ml H₂SO₄ (N free) and the catalytic mixture (K₂SO₄ and CuSO₄ in 5:1 ratio) and kept for digestion. The digested material was transferred into volumetric flask and volume made upto 50 ml. A suitable aliquot (5 ml) was taken into distillation apparatus along with 5-10 ml 40% NaOH. Ammonia was collected in 4% boric acid containing 2-3 drops of mixed indicator for 7-10 minutes and titrated with N/50 sulphuric acid solution.

\[
\text{Sample tittered – Blank taken \times Normality of H}_2\text{SO}_4 \times 100
\]

\[
\%	ext{ Nitrogen} = \frac{\text{Sample weight}}{\text{Normality of H}_2\text{SO}_4} \times 100
\]

Crude protein

Crude protein content in leaf and stem was determined in form the total nitrogen and multiplied by 6.25 (A.O. A. C., 1960)

Phosphorus

Total phosphorus content in dried samples was estimated as per method described by Kitson and Mellon (1944). One gram material was digested in tri acid mixture (H₂SO₄ + HNO₃ + HClO₄ in 9: 3: 1 ratio). The suitable amount of aliquot was taken in 25 ml volumetric flask with equal amount of metavendate reagent. Volume was made upto 25 ml, and kept for colour development. After colour development reading was taken at 480 mμ and phosphorus is calculated from standard curve.

Potassium and Calcium

These chemical contents were analysed in dried plant material directly by the Digital flame Photometer. One gram powdered plant material was taken in conical flask
and digested in tri acid mixture. A white colourless digested material was filtered using whatman filter paper (No.1) into 100 ml volumetric flask. Suitable aliquot was taken for direct reading in the flame photometer using K and Ca filter separately and actual content were calculated from the standard curve.

**Magnesium**

Magnesium content in leaves was determined by Thiazole-yellow method as described by Johnson and Wrich (1959) and modified by Yadava *et. al.*, (1969). One gram oven dried plant sample taken in crucible and moistened with 2 ml alcoholic sulphuric acid. The sample was kept in muffle furnace at 660 °C for 4 hrs to convert it into ash. The ash dissolved with distilled water and transferred into 100 ml volumetric flask through washing. A suitable amount of aliquot was taken in 50 ml volumetric flask. One ml of 50% Hydroxytamine hydrochloride solution was added. 5ml of 2% starch compensating solution (2 gm starch + 100 ml boiling distilled water, compensating reagent; 3.7 g calcium chloride, 0.7 g aluminium sulphate, 0.36 g magnesium chloride and 0.60 g sodium phosphate in one liter distilled water containing 10 ml HCl. These were mixed in equal amount of starch solution) and one ml of 0.10 % titan yellow and mixed thoroughly then added 5 ml of 2.5 N NaOH solution and volume was made with distilled water and allowed to stand from 10 to 30 minutes. Colour intensity was measured at 525 mµ and the amount of magnesium content was obtained from the standard curves.

**Seed yield and yield contributing characters**

The number of fertile tiller per plant, emerged head population density, panicle length, branch number per head and spikelets per branch were recorded during the second year of experimentation. The seed setting and retention percentage were also recorded. To record seed yield, the seeds were harvested at maturation by repeated hand harvesting/striping. After harvesting all the collected seeds were combined cleaned properly and dried in shade. The seed yield per plot was recorded. For recording pure germinating seeds (PGS) the seeds collected per meter square area was cleaned through sample blower and only heavy filled seeds was collected and noted as
PGS. For taking the seed test weight, thousand seeds of uniform size and colour were counted and seed weight was taken by using mettler balance.

**Seed germination**

The seeds of *Panicum maximum* are very small consisting glumes which possess’ problem in germination and seed remain dormant for some time. To work out the germination rate, the following experiments were conducted.

1. **Effect of pre-sowing seed treatment on germination**

   Pre-sowing seed treatments of GA₃ (100 ppm), KNO₃ (0.2 % and 0.4 %) and dil. H₂SO₄ (2 min.) were given in the seeds collected in September 1998 and stored for 8 months at ambient condition under lab condition. The germination percentage was calculated at different days interval.

2. **Effect of storage period on germination**

   To see the effect of storage period on seed germination, the seeds harvested in the month of September 98 and stored in polythene bags (700 gauze) under ambient condition in the lab of Seed Technology Division were taken. The observations on germination percentage were recorded after 3, 6, 9, 12, 15, 18, and 21 months of storage during 1999 and January 2000.

3. **Speed of germination**

   The seeds harvested in the month of September 98 and stored under ambient condition upto June 1999 were taken to study the speed of germination. The experiment was laid out in July 1999, at room temp in the lab and rate of germination was recorded on alternate days, till constant germination.

4. **Germination studies of the seeds produced by the foliar application of NAA, DAP, K and their combinations**

   The seeds produced by foliar application of DAP and K (2,4 and 6 kg ) and NAA (10, 50 and 100 ppm) either alone and/or in combinations were tested for seed
quality in terms of seed test weight and germination. To see the effect of these treatments on germination, seeds harvested in September 98 and stored in the polythene bag (700 gauze) at ambient condition in the lab were taken. Seed germination studies were carried out in the lab condition in June 99 after eight months of storage. The experiment was laid out in the petri dishes in three replications. The germination percentage was recorded on alternate days till constant germination.

5. Effect of sowing depth on germination

The seeds harvested in the month of Sept. 98 and stored in the polythene bag under ambient conditions in the lab were sown in pots at (0.0, 0.5, 1.0, 1.5, 2.0 and 3.0 cm depth. The pots filled with garden soil and 25 seeds were sown in each pots. Each treatment was replicated thrice under the natural environment prevailing at Jhansi.

6. Effect of temperature on seed germination

Seeds of *Panicum maximum* were treated with 0.01% of mercuric chloride. The experiment was conducted at different temperature treatments 20 °C, 25 °C, 30 °C and 35 °C. Twenty five seeds were put on whatman filter paper in the petridish and replicated thrice. The experiment was maintained in the germinator under controlled temperature (±1 °C). The germination percentage was recorded at alternate days till constant germination.

Statistical analysis and presentation of data

The data were statistically analysed and significant response at 5% levels has been compiled by using the methods of Fisher and Yates (1963) and Panse and Sukhatma (1967). The necessary critical difference (CD) has been worked out for comparison of mean values of various treatments.

Correlation coefficient among various important morphological, physiological and biochemical parameters, assimilatory functions and seed yield were also worked out to establish and inter dependence of characters. Graphical representation has also been made to facilitate easy understanding of the table and responses on the observation.
Figure 1. Meteorological data during 1997 - 99