



**SDI Review Form 1.6**

Journal Name:	<a href="#">Annual Research &amp; Review in Biology</a>
Manuscript Number:	Ms_ARRB_44349
Title of the Manuscript:	<b><math>\beta</math>-Aminobutyric acid raises salt tolerance and reorganizes some physiological characters in <i>Calendula officinalis</i> L. plant</b>
Type of the Article	

**General guideline for Peer Review process:**

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound. To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)



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**PART 1: Review Comments**

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
<p><b>Compulsory</b> REVISION comments</p>	<p><b>In the Keywords:</b></p> <p><i>Keywords: Salt stress; seed priming; proline; flavonoids; lipid peroxidation; antioxidant enzymes.</i></p> <p>The sections are all uppercase. For example:  <b>1. INTRODUCTION</b></p> <p><b>2. MATERIAL AND METHODS</b></p> <p>Suggestions to improve the understanding of the methodology:  <b>2. MATERIAL AND METHODS</b></p> <p><b>2.1 Experimental Site and Growth Conditions</b>                      The experiments were carried out in an greenhouse of Faculty of Science, Taif University, Saudi Arabia (Latitude 21° 26' N and Longitude 40° 29 E, at an altitude of 1200 m), having temperate mild climate. The first experiment was carried out during September-December 2016 and was repeated for the same months in 2017 at the same conditions.</p> <p><b>The data of air temperature and relative humidity are internal or external to the greenhouse? External data were obtained from the station in the study area? Explain it better.</b></p> <p><b>I suggest putting the average data for each experiment.</b></p> <p><b>2.2 Plant Material</b>                      Pot marigold (<i>Calendula officinalis</i> L.) was used in current study as the plant material. Uniform sizes of healthy seeds were selected for seed priming treatment. Seeds were prewashed with Triton X-100 (0.25%) for 1 min to remove uncleanness. Thereafter, seeds were washed twice with distilled water for 2 min and surface-dried. The seeds were kept under shade at 25 °C for 48 h to achieve the content of original moisture [31].</p> <p><b>2.3 Treatments and Experimental Design</b>  <b>The experiments were carried out in randomized complete block design or complete randomized design?</b></p> <p><b>Suggestions</b>                      The experiments were carried out in <b>randomized complete block design or complete randomized design</b> with four treatments and three replicates, each replicate consists of five pots. After washing of the seeds in the subitem 2.2, the seeds were divided into four groups: I) was presoaked in distilled water for 12 h (non-primed seeds as a control); II) was presoaked in 1.5 mM β-aminobutyric acid (BABA) for 12 h in screw cap bottle as seed priming treatment, the BABA concentration was selected from a preliminary experiment; III) was presoaked in distilled water for 12 h and was prepared for salt stress treatment after 45 days from planting; IV) was firstly primed with BABA for 12 h and plants raised from primed seeds were exposed to salt stress treatment.</p> <p>After 45 days from planting, the plants raised from the third and fourth groups were exposed to salt stress with NaCl at 8 dS m<sup>-1</sup> at three days intervals and the soil was washed with tap water every week to prevent salt accumulation during the four weeks treatment period. The salt stress concentration was selected from a preliminary investigation.</p>	



#### 2.4 Crop Conduction and Management

Necessary information's as characterization of the type of soil, sowing procedures. What is the size of the pot? How the volume of water applied was calculated? Drainage system in the pots?

**This text may be moved to this new subitem.**

Control and primed seeds (first and second groups) were irrigated by tap water every three days.

**What is the electrical conductivity of the tap water?**

#### 2.5 Evaluated Parameters

##### 2.5.1 Growth, Yield and Inflorescence

Plant height (cm), branch number, herb fresh ( $\text{g plant}^{-1}$ ) and dry weights ( $\text{g plant}^{-1}$ ) were determined by the end of the experiment. For determination of the leaf area ( $\text{cm}^2$ ), blade area was measured using digital image analysis according to Matthew et al. [57] method. Leaf blade digital image was created in digital format using a Hewlett-Packard scanner (Hewlett Packard, Cupertino, ca), image was scanned at dot/inch (100 dpi), the blade area was measured using public domain software.

During the flowering period, total inflorescence number were recorded and weighted to obtain the total inflorescence fresh ( $\text{g plant}^{-1}$ ) and dry weights ( $\text{g plant}^{-1}$ ). At the flowering stage, the samples were taken for subsequent physiological and biochemical investigations. Immediately after weighing the fresh mass, the material was dried in an oven at temperature of  $70^\circ\text{C}$  for 24 h for inflorescence and by 48 h for plant mass.

##### 2.5.2 Relative Water Content (RWC)

The following relationship as described by Weatherley [58] was used for leaf midday relative water content determination and calculation:

$(W_{\text{fresh}} - W_{\text{dry}}) / (W_{\text{turgid}} - W_{\text{dry}}) \times 100$ , where  $W_{\text{fresh}}$  is the sample fresh weight,  $W_{\text{turgid}}$  is the sample turgid weight after saturating with distilled water for 24 h at  $4^\circ\text{C}$ .

##### 2.5.3 Chlorophyll and Carotenoids Contents

##### 2.5.4 Stomatal Conductance

##### 2.5.5 Proline Content

##### 2.5.6 Hydrogen Peroxide ( $\text{H}_2\text{O}_2$ ) Content

##### 2.5.7 Malondialdehyde Determination (MDA)

##### 2.5.8 Membrane Stability Index (MSI)

##### 2.5.9 Antioxidant Enzyme Activity

##### 2.5.10 Total Phenol Content

##### 2.5.11 Flavonoids Content

##### 2.5.12 Leaf Nutrient Elements

#### 2.6 Statistical Analysis

### 3. RESULTS

**According to subitem's suggestions, you can show the effects for a set of variables with increases or decreases, and do not keep repeating the same discussion for**



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	<p><b>each variable.</b></p> <p><b>3.1 Growth, Yield and Inflorescence</b> This subitem shall contain the information of the Tables 1 and 2.</p> <p><b>The size of the title letters of the tables and their contents must be the same size as the letters of the text. The title should be centralized.</b></p> <p><b>Suggestions. Adjust for all tables.</b></p> <p><b>Table 1. Effects of seed priming with <math>\beta</math>-aminobutyric acid (BABA) on plant height, branch number, leaf area, herb fresh and dry weights of <i>Calendula officinalis</i> L. grown under conditions without and with salt stress</b></p> <p><b>3.2 Leaf Nutrient Contents</b></p> <p><b>3.3 Relative Water Content, Stomatal Conductance, Chlorophyll and Proline Contents, Membrane Stability Index, Malondialdehyde and Hydrogen Peroxide Contents</b></p> <p>These parameters can be incorporated into only a subitem. Placing on a table, not in figures, has to standardize, or one thing or another.</p> <p><b>3.4 Antioxidant Enzyme Activity, Total Phenolic and Flavonoids Contents</b></p> <p><b>In the References</b></p> <p><b>I suggest to review all references carefully, as some are outside the standards of the Journal.</b></p>	
<b>Minor</b> REVISION comments		
<b>Optional/General</b> comments		

**PART 2:**

	<b>Reviewer's comment</b>	<b>Author's comment</b> (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
<b>Are there ethical issues in this manuscript?</b>	<i>(If yes, Kindly please write down the ethical issues here in details)</i>	

As per the guideline of editorial office we have followed VANCOUVER reference style for our paper.

Kindly see the following link:

<http://sciencedomain.org/archives/20>

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