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Simulation of tri-factorial interactive effects on the seed germination behaviors of medicinal plant *Cassia fistula* L.

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ABSTRACT

The effects of sodicity, osmotic water potential {Ψ_s}, temperature and their interaction on the seed germination processes of the medicinal tree *Cassia fistula* was carried out. The present data indicated that, the radicle emergence was greatly affected by the interaction between incubated temperature and sodicity {T × SAR}, but Ψ_s had a non-significant effect. Regardless of sodicity, the optimal temperature produced a maximum value on the germination rate index under relatively high Ψ_s. Conspicuously, the Ψ_s had a predominant role in the elongation of both radicle and hypocotyl. The same effect of Ψ_s on the fresh biomass of embryonic axis organs and storage tissue of seedlings was true, whereas, the temperature effect had a sub- dominant role. The moderate Ψ_s and sodicity levels exerted high biomass accumulation efficiency under the optimal temperature. At the same temperature, the seedling vigor index was reached to the highest value at sodicity level {20%} and moderate Ψ_s. The correlation between the seedling water content and germination characteristics were discussed.

INTRODUCTION

During the invasion and cultivation of hot areas by medicinal and ornamental plants such as *C. fistula*, the seed germination and seedling establishment were exposed to many environmental stresses. A scientific investigation of any economic plants to be introduced to desert areas was of utmost importance [1]. In arid and semi-arid regions, the main stress effects on plant development can be summarized out in the following points: water scarce, atmospheric aridity, salinity, imbalance and toxicity of such ions [2]. Therefore, the germinated seeds and seedling growth were behaved many strategies to overcome the above mentioned stresses. Whereas, the seeds had the highest resistance to unfavorable ecological conditions, the seedlings had a lowest [3]. On the other hand, the single factors were greatly not indicative of their effects on the seed germination, but also their combination with each other. Hence, the treatment combinations of such experimental factors were necessary to evaluate the role of each factor and their interactions depending on the suitable experimental design [4].

The seed germination traits can reflect the environmental conditions under which seedlings can be successfully established [5]. It was found such interaction effects of ecological factors on plant development may change from synergistic to antagonistic or vice versa [6, 7]. In saline areas, the osmotic and ionic stresses interact together to inhibit or delay germination of seeds during radicle emergence [8]. Furthermore, salinity can interact with Temperature to affect the seed germination process. Meanwhile, low osmotic water potential may inhibit germination; the detrimental effect of osmotic stress was generally decelerated under the optimal temperatures of seed germination [9]. Accordingly, the strategies of successful establishment of critical plant stages in dry lands depend on the success of the seed germination process and seedlings growing under the above mentioned adverse conditions [10].

The successful seed germination of the studied plant was depending on the magnitude of resistance of seedlings against the osmotic water potential, sodicity and heat stresses {singly or/and in combinations}. The aim of this investigation was to evaluate the strategies of germination behaviors of *C. fistula* L. Seeds affected by a single factor as salinity, sodicity and temperature or / and their combinations. Parameters of investigating species were mainly focused on those concerned with the main characteristics of germination {Radicle emergence, elongation of embryonic axis, rate of germination, seedling vigor index, allocation of biomass and seedling accumulation efficiency}.

MATERIALS AND METHODS

The investigation was carried out on the seeds of *Cassia fistula* L. {Native species in south –eastern Asia} related to family Fabaceae were collected from the trees grown on the campus of Assiut University, Assiut, Egypt. *Cassia fistula* L. Seeds had naturally impermeable coats cause their physical dormancy. Therefore, special pretreatments were required to attain the proper germination percentage. Seed dormancy was removed by scratching the seed coats with sand paper which was sufficient to ensure nearly full germination. The mature seeds of investigating species were sterilized with sodium hypochlorite solution {5% for 10 min.}, thoroughly washed and embedded in the chemically pure filter paper in sterilized glass Petri dishes {15 cm}. Each dish contained 10 seeds and 20 ml. of treatment solutions {Ψ_s + SAR}, which was found adequate to support the seeds during the period of investigation as the dishes were always covered during incubation.

Adjustments of Simulated Osmotic Water Potential, {Ψ_s} and Sodicity:

The effect of decreased osmotic water potential {Ψ_s} on germination was simulated by using sodium chloride and calcium chloride solutions as substrate media for germinating seeds. The presence of $CaCl_2$ in the incubated media with NaCl could ameliorate the inhibitory effect of Na ion toxicity. Solutions has different water potentials, Ψ_s, were prepared by dissolving certain amounts of sodium chloride {NaCl} and calcium chloride {$CaCl_2$} in water, according to preconstructed calibration curves, with different sodium adsorption ratios {SAR}. The treatment solutions were prepared thus of certain levels of treatment combinations. Seeds were exposed to the following range of osmotic water potentials {Ψ_s}: 0 {control}, -0.3, -0.7, and-1.1 MPa [1]. Another series with SAR {5%; 12.5% and 20%} at the same different levels of osmotic water potential were prepared according to Largerwerrf and Eagle [11]. The highest stress level for each treatment represented the maximum tolerance limit {Least germination %} as revealed by preliminary tests. Sets of 4 Petri-dishes were randomly assigned to each osmotic potential level with {Ψ_s + SAR} or without SAR and then incubated at the specific temperatures as explained before. Seeds incubation was terminated after 15 days of incubation, a period long enough to cover any delay of germination due to Stress especially at low water potential levels or extreme temperature treatments. Therefore, treatment combinations of this investigation were covered the three factors used {osmotic water potential, SAR and temperature}.

Adjustments of Incubation Temperatures:

Incubators with air circulation were used in testing the temperature effect on germination. The incubators were kept constantly dark during the incubation period. The tests were run at: 20°C, 28°C and 36°C.

Measurements and Calculations of Seed Germination Criteria:

At the end of the incubation period, many criteria were recorded at various treatments for successful germination. The parameters of germinated seeds were comprised:

1- Counting the number of germinated seeds {Radicle emergence} calculated as % of the total seeds of the treatment according to El- Sharkawi and Farghali [12].

2-Germination rate index {GRI}:

A maximum germinated seeds in relation to the number of days that the germination occurred were germination rate index {GRI}. The GRI could be calculated {germination % day^{-1}} according to Esechie [13].

3- Elongation of embryonic axis:

The elongation of both radicle and hypocotyl {Epigeal germination} were measured {cm} at different treatment levels.

4-Determination of fresh and dry weights {mg} of seedling organs:

Changes in fresh and dry weights of such seedling organs at various treatments will indicate the degree of building up of materials in the embryonic axis. This was corresponding to depletion from the storage tissue. Therefore, fresh and corresponding dry weights {oven-dry at 80^{0}C} for each organ were determined as percentage from the whole seedling {cotyledon, radicle & hypocotyl} at different treatment combinations.

5-Biomass accumulation efficiency {BE}:
This assessment refers to the biomass building up which was corresponding to the embryonic axis elongation. Therefore, the expressed accumulation efficiency as: mg dry weight cm^{-1} length.

6-True seedling vigor index {SVI}:

This treatment was determined according to Vashisth and Nagarajan [14] as the following equation:

$$SVI = Mean\ of\ seed\ germination\ (\%) \times Mean\ of\ seedling\ length$$

This may not be quite indicative of normal growth or any impairment of seedling growth. Hence, this criterion could be modified according to the equation:

$$SVI = Mean\left(\frac{\%\ of\ seed\ germination\ x}{accumulation\ efficiency\ of\ embryonic\ axis}\right)$$

7-Seedling water content {as %}:
The imbibed water content of seeds for each treatment combination was calculated as a percentage from the fresh weight of the whole seedling by the following equation:

$$Water\ content\ \% = \frac{fresh\ wt. - dry\ wt.}{fresh\ wt.} \times 100$$

Statistical Analysis:

Statistical inferences are necessary to evaluate the effects and relative roles {shares} of single factors and their interactions on germination phases. The significant effects of single factors and their interactions were determined by analysis of variance [15]. Based on the significance status, the magnitudes of the relative effect of every single factor and its interaction was determined as a percentage by using the SPSS program {Share percent} [16], which is considered a test used to indicate the degree of control of each factor and its interaction on the tested parameters according to Ploxinki, [17], and applied by El-Sharkawi and Farghali [12]. A simple linear correlation coefficient {r.} between the investigated parameters was tested according to Ostle [15].

RESULTS

The type of seed germination in *C. fistula* was epigeal and the different characteristic parameters were comprised:

1. Radicle Emergence {Germination %} and Germination Rate Index {GRI}:

The radicle emergence of germinated seed {as %} was illustrated in figure {1}. This percentage was an expression of the success of radicle under Ψ_s, temperature, sodicity{SAR}, and their combinations. Regardless of sodicity, the temperature range 20-28 ^oC exerted a boosting effect on seed germination at different Ψ_s levels. Meanwhile, the supra- optimal temperature {36 ^oC} was decelerated this process, particularly under low Ψ_s. Statistically; temperature, sodicity and their interaction had highly significant effect on the radicle emergence. Although, the interaction {T x SAR} had predominant role {36.8%} on the radicle emergence, single factors had a subsidiary role {Table, 1}. This means that, the radicle emergence was adapted to Ψ_s and sensitive to both SAR and temperature. The germination rate index {GRI} which refers to the speed of seed germination was affected by the investigated factors {Figure, 1}. In general, the optimal temperature {28 ^oC} induced an increase in GRI, especially under high Ψ_s levels {0 to -0.3 MPa}. Commonly, the GRI in *C. fistula* was decreased in response to reduced water potential and supra- optimal temperature. At different SAR levels, this species reflects mesomorph characters where a maximum GRI tended 3.3 at high Ψ_s and optimal temperature.

2. Elongation of Embryonic Axis Organs:

The elongation of both radicle and hypocotyl seemed to be different in their responses towards the experimental factors {Figure, 2}. Under optimal temperature a high sodicity {20% level} exerted a maximum radicle elongation {2.3cm} at high Ψ_s level and, while the hypocotyl length was reached to the highest value {5.2cm} at SAR levels from 5 to 12.5%. The elongation pattern of both organs was similar to each other with supra- temperature and low Ψ_s levels. Also, moderate levels of SAR {12.5%} had a boosting effect on the radicle elongation under low temperature and salinity stress. The F values {Table, 1} indicated that, the Ψ_s, temperature and their interaction had highly significant effect on both radicle and hypocotyl elongation. Meanwhile, Ψ_s played a major role in both organ elongations {Sharing 52.1 and 50.4%, respectively}; the temperature had a secondary role {23.5 and 34.7%, respectively}.

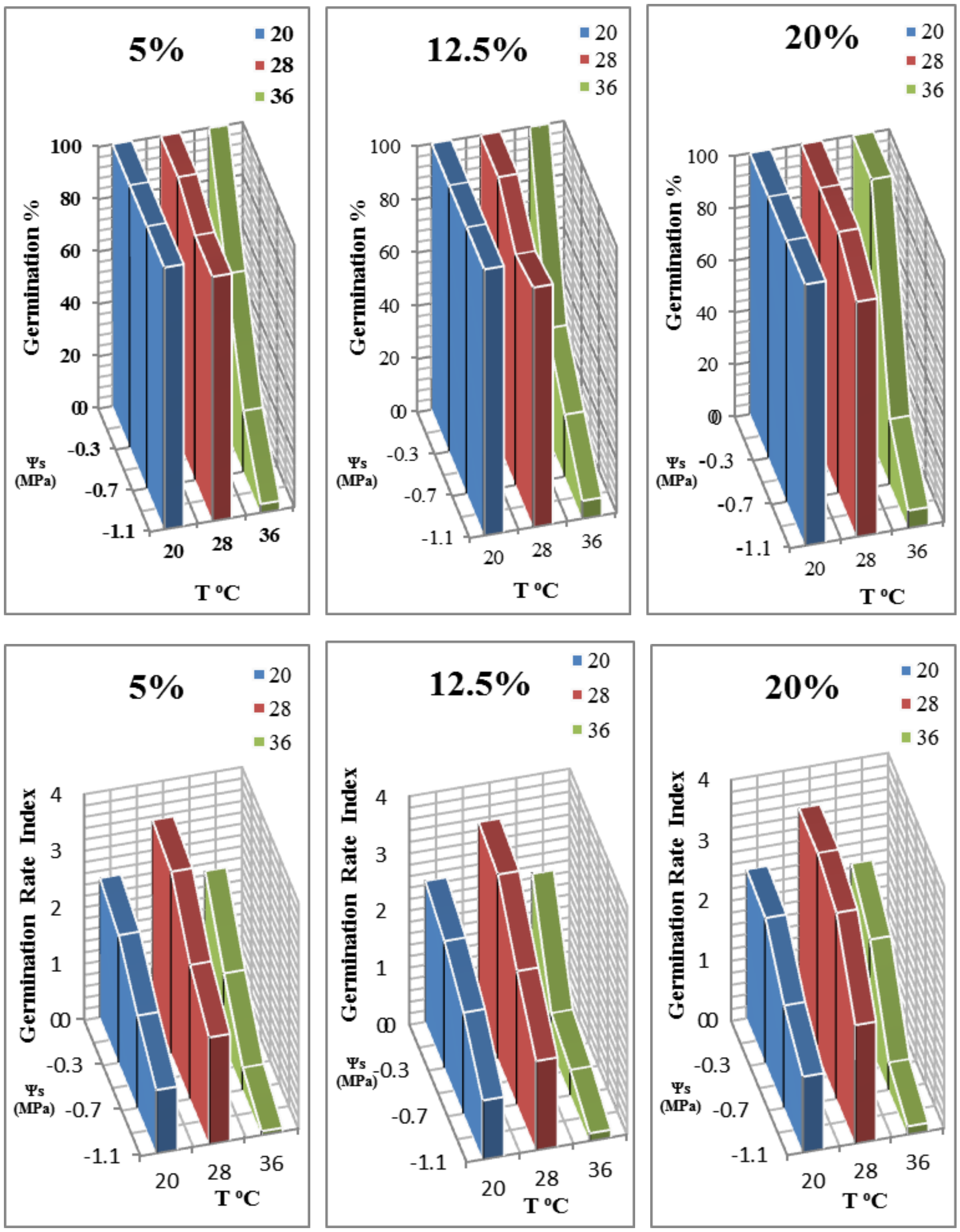

Figure 1: Average values of germination % and germination rate index of seeds in *Cassia fistula* plant at different osmotic water potential {Ψ_s}, temperature {T ^oC} and sodicity {SAR} levels.

Table 1: F values and sharing % of different germination characteristics in *C. fistula* plant under the effects of osmotic water potential {Ψ_s}, temperature {T ^{o}C}, sodicity {SAR} and their interactions.

Parameters / Source of variance		Germination %		Radicle Length.		Hypocotyl Length	
	df	F	Share (%)	F	Share (%)	F	Share (%)
Ψ_s	3	1.24	5.5	29.66**	52.1	39.23**	50.4
T	2	8.05**	23.9	20.09**	23.5	40.53**	34.7
SAR	2	8.15**	24.2	1.05	1.2	0.08	0.1
Ψ_s * T	6	0.55	4.9	4.59**	16.1	4.58**	11.8
Ψ_s * SAR	6	0.28	2.5	0.65	2.3	0.08	0.2
T * SAR	4	6.20**	36.8	0.63	1.5	0.40	0.7
Ψ_s * T * SAR	12	0.12	2.2	0.48	3.4	0.40	2.1

*Significant at 0.05 confidence level **significant at 0.01 confidence level

Table 1: continued

Parameters / Source of variance		Radicle Weight		Hypocotyl Weight		Cotyledon Weight	
	df	F	Share (%)	F	Share (%)	F	Share (%)
Ψ_s	3	28.37**	33.8	68.64**	44.4	94.03**	51.7
T	2	38.19**	30.3	95.17**	41.0	102.09**	37.4
SAR	2	6.67**	5.3	2.47	1.1	1.85	0.7
Ψ_s * T	6	3.16**	7.5	6.74**	8.7	6.09**	6.7
Ψ_s * SAR	6	1.56	3.7	0.41	0.5	0.31	0.3
T * SAR	4	7.09**	11.3	1.93	1.7	1.57	1.1
Ψ_s * T * SAR	12	1.712	8.1	1.03	2.7	0.94	2.1

*Significant at 0.05 confidence level **significant at 0.01 confidence level

3. Fresh Biomass of Seedling Organs:

The allocation of biomass {Fresh weight} between seedling organs at different treatment levels {Figure, 3} indicated that, the SAR 12.5% had a boosting effect on the fresh weight of hypocotyl under high Ψ_s levels {from 0 to -0.3MPa} and optimal temperature {28 ^oC}. This improving effect achieved 55.8% of the whole seedling weight. At similar conditions of both {SAR and Ψ_s}, the highest translocate biomass to the radicle {15.0% of whole seedling} was noticed under sub-optimal temperature. Remaining food material in the storage tissue was observed at low Ψ_s levels and supra-optimal temperature.

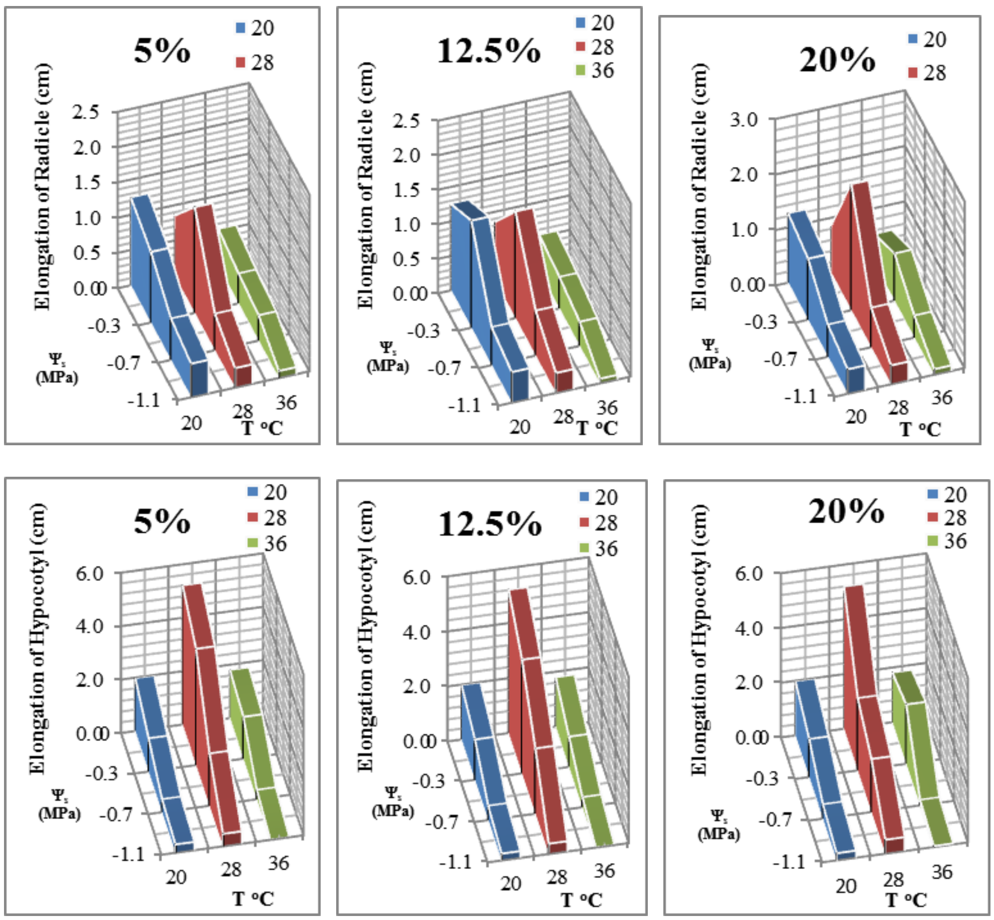

Figure 2: Average values of radicle and hypocotl elongation of *Cassia fistula* plant at different osmotic water potential {Ψ_s}, temperature {T ^oC} and sodicity {SAR} levels.

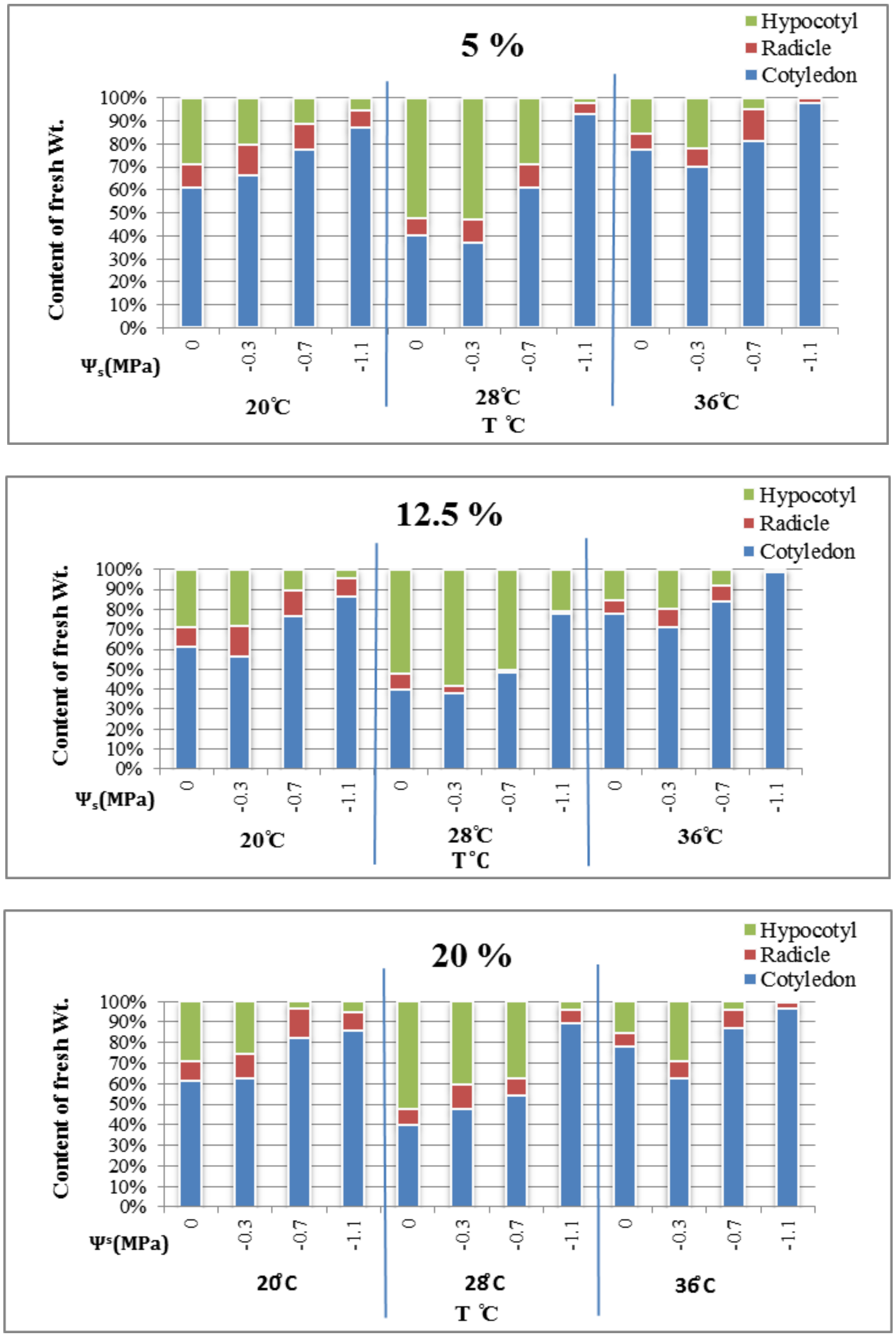

Figure 3: Average values of fresh weight {as %} in different seedling organs of *Cassia fistula* plant at different osmotic water potential {Ψ_s}, temperature {T ^{o}C} and sodicity {SAR} levels.

Commonly, the hypocotyl was gaining a high percentage of fresh biomass among various seedling organs. F values {Table, 1} indicated that, the different investigated factors $\Psi_{s,}$ SAR ,T and their interactions had a highly significant effect {With some exceptions} on the radicle fresh weight. Temperature, Ψ_s and their interaction had similar roles on the fresh biomass of the rest seedling organs. The share percentage of each factor emphasized that, Ψ_s had a dominant role on the fresh weight of biomass in radicle, hypocotyl and storage tissue {33.8%, 44.4% and 51.7%, respectively}, whereas the temperature had a sub- dominant role {30.3, 41.0, and 37.4, respectively}.

4. Biomass Accumulation Efficiency {BE}:

The true growth of the embryonic axis was released by the degree of dry matter building up per length of seedling which is indicative of normal growth {Expressed as accumulation efficiency}. The BE of *C. fistula* seedling under experimental factors {Figure, 4}, revealed that, the SAR 20% induced the highest biomass efficiency {33.2 mg dry Wt. cm $^{-1}$ } at optimal temperature and a wide range of Ψ_s levels{0 to -0.7MPa}.While, the supra-optimal temperature {36 ^{o}C} had a boosting effect on the BE under high Ψ_s level{-0.3MPa}. Under sub-optimal temperature, the BE values were decreased at different levels of Ψ_s and SAR. Regardless of Ψ_s, the best true growth of *C. fistula* was under high SAR level and optimal temperature.

5. Seedling Vigor Index {SVI}:

The vigor index {Figure, 4} was an expression of plant health and grown state. Therefore, a high value of SVI indicated good seedling viability. It was found that, the optimal temperature induced an increasing effect in SVI of *C. fistula* and produced the highest value {28.0} under Ψ_s=-0.3MPa and SAR level 12.5% { Figure, 4}.The same increasing response in SVI {31.0} were shifted to Ψ_s=-0.7MPa and SAR 20% level. Under supra-optimal temperature {36 ^{o}C} a peak was shown at low water stress and high SAR level. The effect of sub-optimal temperature exerted moderate SVI at different levels of Ψ_s and SAR.

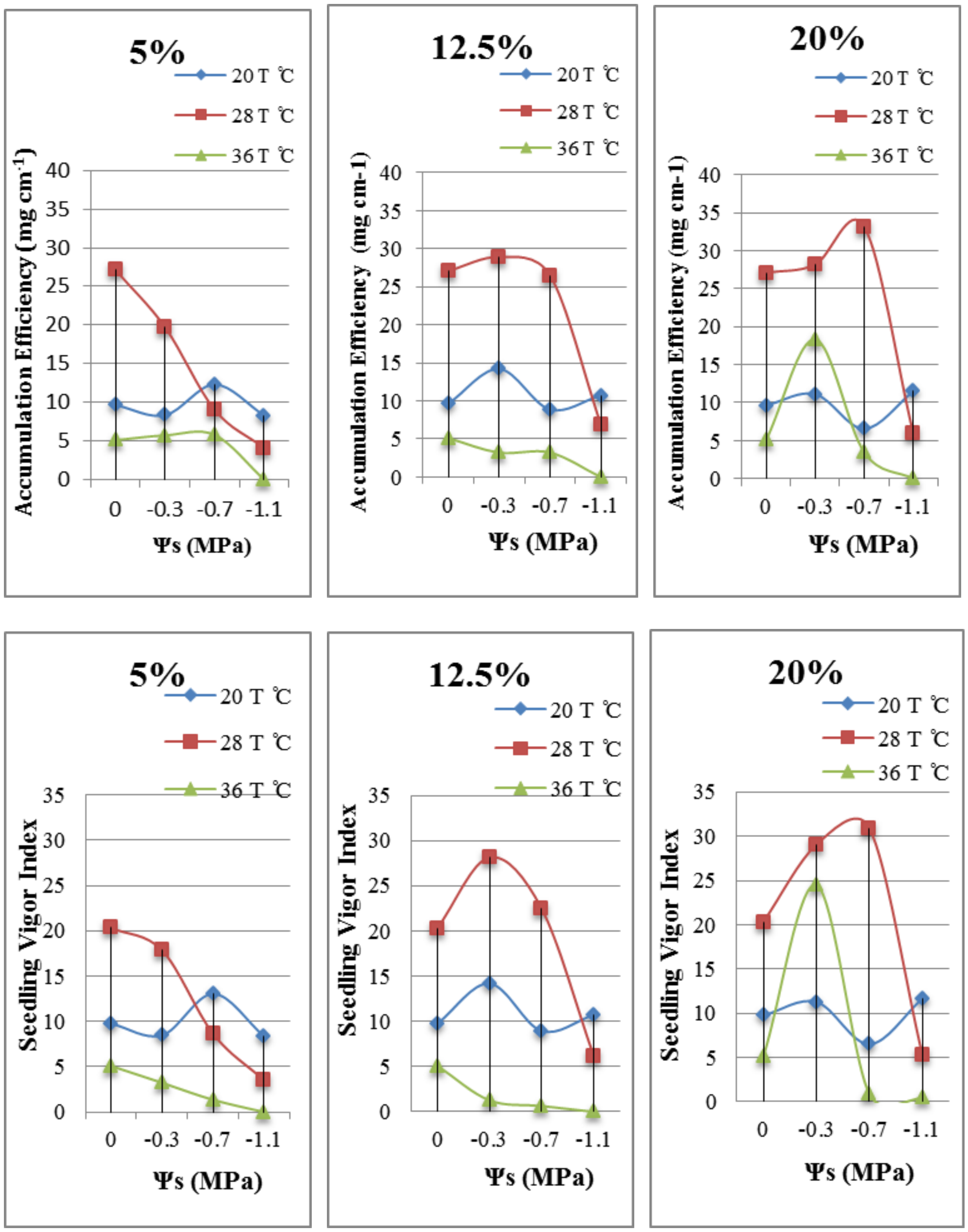

Figure 4: Average values of biomass accumulation efficiency and {mg cm^{-1}} vigor index of seedling in *Cassia fistula* plant at different osmotic water potential {Ψ_s}, temperature {T ^{o}C} and sodicity {SAR} levels.

6. Seedling Water Content:

The successful seed germination was mainly depended on the amount of imbibed water by seeds. In the whole seedling, the water content was ranged between 72.1 and 96.3% which was stimulated by the presence of mucilaginous substances inside the seed coat {Figure, 5}. Conspicuously, the seedling had higher water content under low Ψ_s levels than that under control. Also, a supra-optimal temperature had a boosting effect on the water content of *C. fistula* seedlings at a wide range of Ψ_s and SAR levels. This may be due to the increasing of cells osmolality in response to the temperature level increases.

The various germination characters such as germination percentage, GRI, SVI and biomass accumulation efficiency were independent {non-significant} on the seedling water content under the effect of single factors {Table, 2}. While the bi- and tri- factorial interactions were exerted a significant negative correlation {with some exceptions} on the previous parameters. Under {Ψ_s x T x SAR} and {T x SAR} interaction, a significant negative correlation was observed between water content and all above mentioned parameters of *C. fistula* seedlings {except in the case of bi-factorial interaction with germination %}. Whereas, the {Ψ_s x SAR} interaction exerted the same correlation between water content with germination% and GRI, whereas, the negative correlation was detected under {Ψ_s x T.} interaction in the case of GRI.

DISCUSSION

The presence of sodium ions in plant root media was necessary, but the excess of these ions caused a toxicity which has deleterious effects on the plant growth [18]. Therefore, the seed germination and early seedling establishment success in the saline habitats refer to the magnitude of temperature, salinity and specific ions such as sodium tolerance, particularly in the arid and semi-arid regions. In this investigation, obtained data indicated that the germination traits of the medicinal plant *Cassia fistula* were greatly affected by the previously mentioned factors and their interactions. Conspicuously, the high sodicity level {20%} exerted the highest radicle emergence under high Ψ_s and sub-optimal temperature {Figure, 4}. Furthermore, the F value indicated that the temperature, sodicity and their interaction had highly significant effects on the radicle emergence.

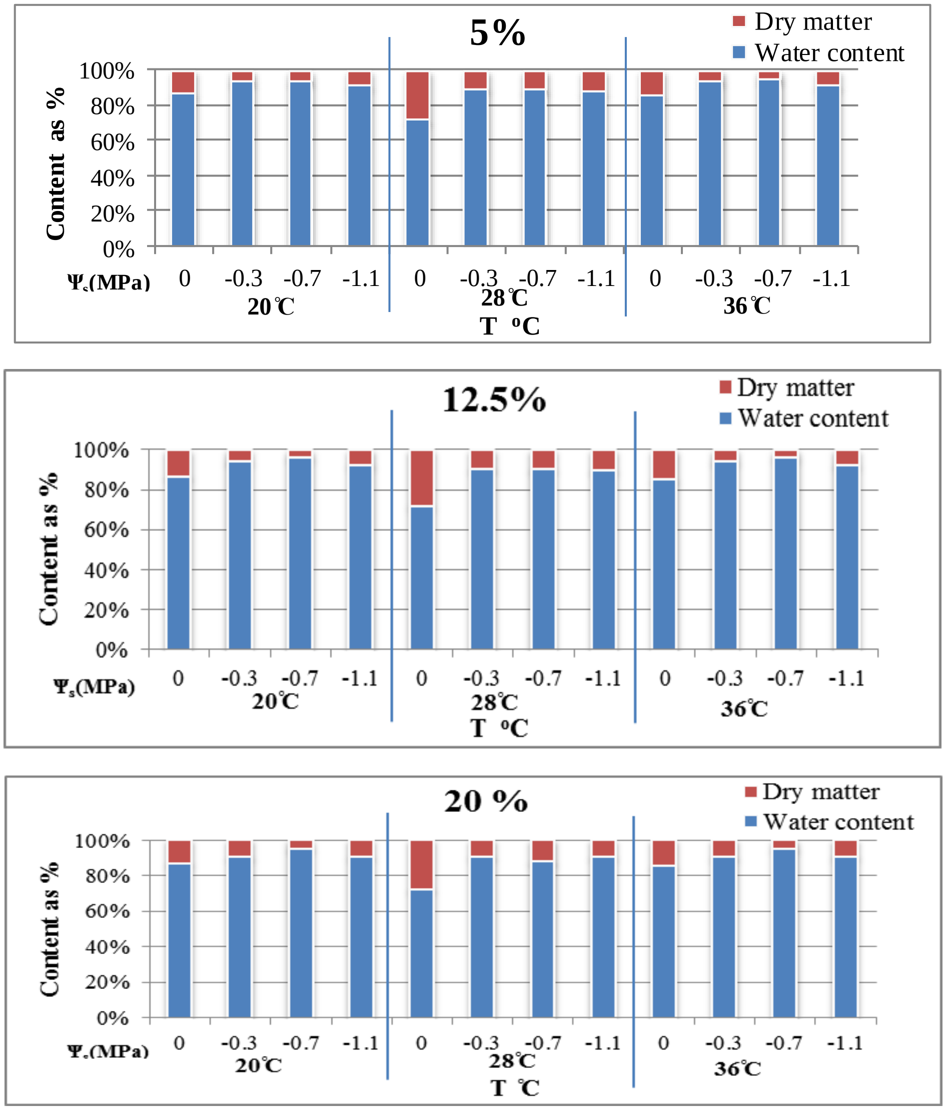

Figure 5: Average values of seedling dry matter and water content {as %} in *Cassia fistula* plant at different osmotic water potential {Ψ_s}, temperature {T ^{o}C} and sodicity {SAR} levels.

Table 2: Correlation coefficient {r.} between seedling water content and germination characteristics in *C. fistula* plant under the effects of osmotic water potential {Ψ_s}, temperature {T ^{o}C}, sodicity {SAR} and their interactions.

Parameter / Source of variance	*Percentage of Germination*	*Germination Rate Index*	Seedling Vigor Index	Accumulation Efficiency
Ψ_s	-0.734	-0.659	-0.075	-0.223
T	-0.399	-0.835	-0.871	-0.924
SAR	-0.942	-0.928	-0.337	-0.182
Ψ_s * T	-0.345	-.617*	-0.413	-0.537
Ψ_s * SAR	-0.834**	-0.816**	-0.263	-0.417
T * SAR	-0.409	-0.826**	-0.774*	-0.837**
Ψ_s * T * SAR	-0.354*	-0.615**	-0.385*	-0.501**

*Significant at 0.05 confidence level **significant at 0.01 confidence level

The interaction {T x SAR} had predominant role {sharing=36.8%}, whereas the single factor {SAR} had the secondary role {24.2%} on the radicle emergence. This means a prominent effect of such interaction on radicle emergence and its sensitivity to sodicity under temperature change. The Ψ_s had a non – significant effect as a result of the presence of mucilaginous substances {polysaccharides} in the seed coat which accelerates the imbibition seed force against low osmotic water potential and indicated the adaptability of the germination process in *C. fistula* to salinity [19]. Consequently, the seedling water content was reached 72.1% at the control and 96.3% under salinity stress may be due to the accumulation of osmotic solute [20]. Accordingly, the germination rate index of seeds at different SAR levels tended to a maximum {3.3} under control and moderate temperatures.

Radicle length was an important absorbing organ, whereas hypocotyl elongation played a crucial role through the utilization of radiant energy by raising plumule [12]. Hence, the effects of osmotic and specific ion toxic inhibit the maintenance of nutrient levels essential for plant growth, consequently limiting root emergence and seedling growth [21]. Apparently, as in case of emergency, the highest sodicity produces a higher radicle elongation {2.3cm} under the optimal temperature, while this temperature stimulating effect was exerted the highest hypocotyl elongation {5.2cm} at moderate SAR level under low salinity stress. In general, the decreased Ψ_s and supra-optimal temperature delay the elongation of both embryonic organs. The analysis of variance emphasized that, $\Psi_{s,}$ temperature and their interaction had a highly significant effect on radicle and hypocotyl elongation, the Ψ_s had a major role {52.1% and 50.4%, respectively} and temperature had the sub- dominant role. This agreed with data obtained by El- Sharkawi *et al*. [22, 23]. This reflected the magnitude of adaptive aspects to the seedling establishment under severe conditions of both edaphic and climatic stresses.

The fresh biomass of both radicle and hypocotyl {Figure, 3} were variable in their response to the investigated factors. In saline lands where soil water was scarce, plants usually allocate more biomass to roots to increase water uptake [24]. It was quite clear that, the low and high SAR had a boosting effect on the fresh biomass of radicle at sub-optimal temperature and moderate Ψ_s, while the moderate SAR produce high fresh biomass under optimal temperature and relatively high Ψ_s levels. Statistically, {Table, 1} the fresh weight of the radicle was greatly affected by single factors and their combination {Ψ_s x T and T x SAR}. Only, Ψ_s, temperature and their interaction had highly significant effect on the fresh biomass of hypocotyl. Generally, the osmotic water potential had a major role on both organs {sharing 33.8% and 44.4%, respectively}, While the temperature has a secondary role. Under extreme factors of Ψ_s and temperature, the fresh biomass of storage tissue was conserved. Also, both factors and their interaction had a significant effect, where Ψ_s and the temperature had dominant and sub-dominant roles on the depletion of storage tissue biomass {51.7% and 37.4%, respectively}. On the other hand, the SAR range 12.5 -20.0% has a boosting effect and produced the highest value {33.2mg cm^{-1}} of seedling biomass efficiency {Figure, 4}. This value was detected under optimal temperature and moderate osmotic water potential level {-0.7MPa}. This means that the true growth of *C. fistula* was affected by the presence of sodium ions in the rooting medium.

Similarly, the seedling vigor index {SVI} was more responded to the same conditions of accumulation efficiency, which referred to a healthy and viability of the seedling growth [25] found a high reduction in vigor index as salinity level increasing. Apparently, the optimal temperature stimulating the seedling vigor index in *C. fistula* was tending to a maximum {31.0} under moderate salinity stress and relatively high SAR level {Figure, 4}. This emphasizes that, the sodicity had an induced effect on SVI of *C. fistula* seedlings under optimal and supra-optimal temperatures, particularly at reduced water potential levels {-0.3 and -0.7MPa}. This might provide an ecological importance and high viability potential of a seedling establishment grown in saline and hot lands.

The correlation between seedling water content and the calculated parameters: germination percentage, GRI, SVI and biomass accumulation efficiency were independent under the effect of single factors. However, the bi- and tri-factorial interactions produced a significant negative correlation between the water content and these parameters, where the tri- factorial interaction predominates among the investigated factorial interactions. This implied that, the water functions during seed germination processes of *C. fistula* were controlled by the interactions between the experimental factors.

CONCLUSION

It was apparent that, the germination traits in *C. fistula* seeds had a variable response under the investigated factors singly or in combinations. Meanwhile, the percentage of germination was mainly affected by the interaction between temperature and SAR, the rest parameters were dominantly affected by the single factor Ψ_s. Many strategies could be exerted by seeds of *C. fistula* against thermal, reduced water potential, sodicity and their interactions such as high germination percentage and germination rate index as a result of imbibition force increases. Additionally, the stimulation of biomass accumulation efficiency, consequently high seedling vigor index under relatively moderate SAR levels and optimal temperature. Finally, the true growth of seedlings was reflected as increment in both the biomass accumulation efficiency and seedling vigor index under cultivation of xeric habitat conditions.

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