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盐碱混合生态条件的人工模拟及其对羊草胁迫作用因素分析

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摘要:将中性盐 NaCl 和 Na_2SO_4、碱性盐 $NaHCO_3$ 和 Na_2CO_3 按不同比例混合,模拟出 30 种盐度和 pH 各不相同的盐碱生态条件,并对羊草苗进行盐碱混合胁迫处理。测定其日相对生长率(*RGR*)等 7 项胁变指标,用数学方法分析盐度、缓冲量等各种胁迫因素与诸项胁变指标间的相互关系。结果表明:30 种处理均匀覆盖了总盐度 50~350 mmol/L,pH 7.14~10.81 范围内的各种盐碱条件。用盐度、缓冲量、pH 和[Cl^-]即可代表盐碱混合胁迫的所有胁迫作用因素。诸胁变指标与这 4 因素间均具有高度线性相关性。4 因素对胁变的贡献明显不同,其中缓冲量和盐度是决定性的主导因素,pH 和[Cl^-]的作用明显次之,有时甚至可以忽略。不同胁变指标与各因素的关系也有所不同。分析结果表明:对于盐碱混合胁迫来说,以盐度加缓冲量代表总胁强较为合理。

关键词:盐胁迫;碱胁迫;胁迫因素;盐度;缓冲量

A Simulation of Salt and Alkali Mixed Ecological Conditions and Analysis of Their Stress Factors in the Seedlings of *Aneurolepidium chinense*

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Abstract: According to the salt components in extent alkaline soil in the west of Jilin Province of China, two neutral salts NaCl and Na_2SO_4 and two alkalic salts $NaHCO_3$ and Na_2CO_3 were selected for this study. The selected salts were mixed in various ratios according to the varying ranges of salinity and pH in the natural soil and the tolerability of *Aneurolepidium chinense* (Trin.) Kitag to the salt-alkaline stress. Six treatment groups (labeled as A, ... ,F) with gradually increased proportion of alkalic salt were set. And in each group, five concentration treatments were 50, 125, 200, 275 and 350 mm respectively. The sum total was 30 combinations with different salinity and pH.

Seeds of *A. chinense.* were collected from a natural grassland located in the west of Jilin Province of China and were sown in 17 cm (*D*). plastic pots of washed sand. All pots were put outdoors and artificially kept out of rain. The seedlings were grown under natural conditions by the sand culture method, and sufficiently watered with Hoagland nutrient solution every two days. Evaporated water was replenished with distilled water at other times. Each pot retained 40 plants.

When the seedlings were 4 weeks old, they were subjected to stress. The seedlings growing uniformly (96 pots) were selected and randomly divided into 32 portions with 3 pots per portion (3 replications). Among the 32 portions, 1 portion was a control (CK); 1 portion was used to determine the growth index

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at beginning treatment; others 30 portions were various stress treatments and were labeled as A1, A2... F5 respectively. Control plants were maintained by watered with nutrient solution; various stress treatments all took nutrient solutions containing stress salts as the treatment solutions. Stress treatments were performed at 4:00～5:00 p.m., by watering the plants thoroughly with 500ml of treatment solution, divided 3 times for a pot. From next morning, the amount of evaporated water was determined by weighing and the evaporated water was replenished with distilled water every day.

Sampling was taken after 7 d of treatment. The physiological indices of *RGR* (the relative growth rate), tillers, rhizomes, electrolyte leakage rate in leaves and the contents of Na^+, K^+, and proline in shoots were determined. Taking the *RGR* of control as 100%, the percentages of the *RGR* of various treatments relative to control were calculated. The pH values of various treatment solutions were determined with a digital pH meter. The buffer capacity was equal to the millimole amount of H^+ needed to make the pH of 1 L treatment solution dropped to equal with the control by titrating with HCl.

The data obtained all were the average of 3 replications. A statistics analysis on correlation coefficient and multivariate regression was performed by using a program Microsoft Excel.

A regular changing of pH values was shown in 30 salt combinations. The pH values increased from group A to group F with increasing alkali salt proportion. Within a treatment group, pH values increased with increasing total salt concentration. The range of pH values among groups is greater than within a group. In consideration of the main toxic ion Na^+, its concentration increased as 112.5 mmol/L per concentration treatment; the Na^+ concentrations are 75, 187.5, 300, 412.5, and 525.5 mM corresponding to the five salt concentrations in a treatment group. In consequence, 30 salt-alkaline conditions with different salinity and pH were established. Their salinity coverage is from 50 mmol/L to 350 mmol/L; [Na^+] coverage is from 75 mmol/L to 525 mmol/L; pH coverage is from 7.14 to 10.81.

Because the salt component, salinity, and pH in the 30 simulated salt-alkaline conditions were similar with the conditions in natural salt-alkaline soil, these simulated salt-alkaline conditions reproduced the natural complex salt-alkaline conditions. The way to establish complex salt-alkaline conditions was a practicable method for researching complex salt-alkaline stress.

The plants of E5, F4, and F5 three groups all died after stress treatments because the stress strengths were over their tolerability.

The buffer capacity had the greatest total correlation degree between stress factors and seven strain indices, which indicated that alkalic salt was determinant in causing the strains. According to the results of correlation analysis, four stress factors salinity, buffer capacity, pH, and [Cl^-] probably could represent all the stress factors.

Above four stress factors were taken as independent variables, X_1=buffer capacity, X_2=salinity, X_3=pH, and X_4=[Cl^-]. The strain indices were taken as dependent variables i.e. $Y=RGR$ and so on. Multivariate regression analysis was performed for each strain index using the formula $Y=a+b_1X_1+b_2X_2+b_3X_3+b_4X_4$. The importance of each stress factors was compared according to their standardized regression coefficients (b'). The effects of regression were estimated by the coefficient of determination (R^2).

The results showed that there were perfect linear correlations between every strain index and the four stress factors. The effects of the four stress factors on the strain indices were significantly different. Buffer capacity and salinity were dominant factors for all strain indices; pH and [Cl^-] were significantly less important and might be neglected in some cases. The relationships between different strain indices and various stress factors were also different. It is reasonable to consider the salinity plus buffer capacity as the

strength value of salt and alkali mixed stress.

Key words: salt stress; alkali stress; stress factor; salinity; buffer capacity

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欲研究某一胁迫作用的机理,首先要明确这一胁迫所涉及到的所有对植物体具有胁迫效应与植物胁变反应有关的作用因素。关于中性盐(如 NaCl)胁迫的作用因素通常认为有:盐浓度所决定的渗透效应、以 Na^+ 等有害离子所决定的离子效应[1]以及盐离子与其他营养离子间相互作用等[2,3];若是碱性盐(如 Na_2CO_3)则应增加高 pH[4~7]及与此有关的磷、铁营养缺乏等效应[8,9]。在中碱性混合盐的情况下,又发现缓冲量对胁变有极重要的决定性作用[10]。盐碱混合胁迫所涉及的作用因素更多更复杂,要想深入探讨其胁迫作用特点与机理,首先要弄清其作用因素及各因素的地位以及各因素间的相互关系。

由于土壤盐化与碱化往往相伴发生,所以长期以来人们将土壤可溶性盐分的增加笼统地称为"土壤盐碱化"。事实上,由 Na_2CO_3、$NaHCO_3$ 等碱性盐所造成的土壤碱化问题可能比由 NaCl、Na_2SO_4 等中性盐所造成的土壤盐化问题更加严重,在全球大约 $1.5\times19^9hm^2$ 的土地中有 23%的盐土(Saline)和 37%的苏打土(Sodic)[11],在我国,东北草原上的碱化草场已达 70%以上。研究证明:中性盐胁迫与碱性盐胁迫实际是两种性质不同的胁迫,应该将前者称为盐胁迫将后者称为碱胁迫。碱胁迫比盐胁迫具有更大的生态破坏力[12~14]。对于内陆盐碱地来说,多是既含有中性盐又含有碱性盐,所以,盐碱混合胁迫才是实际存在的主要问题。然而从目前有关植物抗盐生理的研究现状来看,却仍然以 NaCl 为重要对象[15],以 Na^+ 离子代谢[16]、抗盐性相关基因的分子生物学[17,18]及盐胁迫信息传导[15]等为主要研究方向。虽然在认识植物抗盐机制方面有了长足进展,但在碱性盐胁迫及盐碱混合胁迫方面,除了作者的研究工作外很少有人涉及。此外,天然盐碱土壤所含盐分的种类、比例、数量等各不相同,甚至同一块盐碱地的不同部位,由于其盐分组成的不同使其盐度和 pH 表现出明显差异,正是这一复杂性使研究受到限制。为了揭示盐碱混合胁迫对植物的作用,本文将两种中性盐 NaCl 和 Na_2SO_4 及两种碱性盐 $NaHCO_3$ 和 Na_2CO_3 按不同比例混合,模拟出 30 种盐度和碱度各不相同的盐碱条件,以此对羊草进行盐碱混合胁迫处理。旨在创建一个研究盐碱混合胁迫的手段,并以此探讨盐碱混合胁迫的主要作用因子及其与植物胁变之间的关系。

1　材料和方法

1.1　盐碱混合胁迫模拟设计

根据吉林省西部盐碱地的盐分组成[19],选定两种中性盐 NaCl 和 Na_2SO_4 和两种碱性盐 $NaHCO_3$ 和 Na_2CO_3,又根据其盐度与 pH 复杂多变的特点以及羊草对盐碱的耐受能力,将 4 种盐按不同比例混合。按碱性盐比例逐步增大的顺序分成 A、B、C、D、E 和 F 等 6 组。每一组内又设有 50、125、200、275 和 350 (mmol/L)等 5 个不同浓度处理。总计为 30 种盐度和 pH 各不相同的盐碱组合。

表 1　各处理所含盐分及其摩尔比

Table 1　Salt composition and its molar ratio of various treatments

处理组 Treatment group	盐组成与摩尔比 Salt composition and molar ratio			
	NaCl	Na_2SO_4	$NaHCO_3$	Na_2CO_3
A	1	1	0	0
B	1	2	1	0
C	1	9	9	1
D	1	1	1	1
E	9	1	1	9
F	1	1	9	9

1.2　材料培养

羊草(*Aneurolepidium chinense* (Trin.) Kitag.)种子采自吉林省西部草原,播于直径 17cm 盛有洗净细砂的塑料花盆内。花盆置于室外,人工遮雨,于自然条件下用砂培法培养羊草苗。每 2d 用 Hoagland 营养液透灌 1 次。其余时间用蒸馏水补充蒸发所失水分。每盆留苗 40 株。

1.3　胁迫处理

苗龄 4 周时,选取长势均匀的羊草苗 96 盆随机分成 32 组,每组 3 盆为 3 次重复。其中 1 组作为对照,1 组用于测定处理开始时的生长指标,其余 30 组为不同处理,依次标为 A1,A2,…,F5。其中 A1 即表示 A 组中总盐浓度为 50 mmol/L 的处理,依此类推。处理于 17:00~20:00 时进行。以含有相应浓度混合盐的营

养液为处理液，每盆500ml分3次透灌花盆。对照只灌完全营养液。从次日起每天用称重法测定每盆失水量，并用蒸馏水补充。

1.4 胁变指标测定

胁迫处理7d后取样，测定各处理的日相对生长率（*RGR*）、分蘖率、根茎数、茎叶Na^+含量、茎叶K^+含量、茎叶脯氨酸含量和叶片电解质外渗率等7项胁变指标。按下面公式测定*RGR*，并以对照的*RGR*为100%，各处理的*RGR*以相对于对照的百分比表示。其余胁变指标的测定参照文献[10]进行。

$$RGR = (\ln \text{处理前生物量} - \ln \text{处理后生物量}) / \text{处理天数}$$

1.5 胁迫因素分析

各处理液的pH值用数字pH计测定。各处理的总盐浓度及Na^+、Cl^-、SO_4^{2-}、HCO_3^-、CO_3^{2-}等离子的浓度 均根据处理液的实际盐分组成算出。本实验所用缓冲量的定义与文献[10]相同，即指用滴定法使1L处理液的pH降至与对照相等时所需H^+的mmol数，并按文献[10]的方法测定。所有实验数据均为3次重复的平均值。用微机进行相关系数、多元线性回归等统计分析。

2 结果

2.1 各处理液的盐度与pH

从图1可见各处理的pH呈明显的规律变化，由于碱性盐的比例依次增加，从A组到F组pH递增。在每一处理组内随盐浓度的增大pH也递增。组间的变化大于组内的变化。若从主要致害离子Na^+的浓度考虑，每组内5个浓度处理的[Na^+]以112.5 mmol递增，依次为75、187.5、300、412.5、525 mmol/L。这就模拟出了盐分组成、pH及其变化规律均与天然盐碱地相似的30种各不相同的盐碱条件，其盐度覆盖从50 mmol/L到350 mmol/L，[Na^+]从75 mmol/L到525 mmol/L，pH覆盖从7.14～10.81。

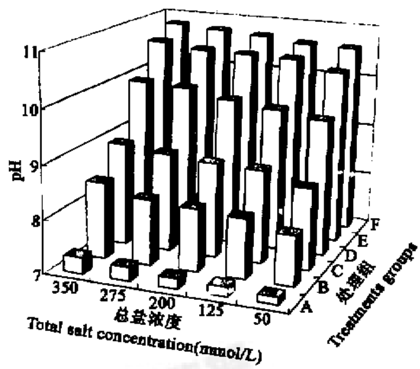

图1 各处理的总盐浓度和pH值

Fig. 1 Total salt concentration and pH of various treatments

2.2 致胁变因素及胁变值数据

胁迫处理后E5、F4、F5等3组材料因胁强超出其耐受范围而全部死亡。剩余27组处理的有关数据列于表2和表3中。

2.3 各致胁变因素与各项胁变指标间的相关性

为明确各胁迫因素与不同胁变指标的关系，首先对二者间进行了相关系数计算。计算结果列于表4。

查相关系数显著性检验表可知：自由度$n=27$时，$r_{0.05}=0.381$（相关系数显著）、$r_{0.01}=0.487$（相关系数极显著）。从表4可见缓冲量、盐度、[Na^+]和[CO_2^{2-}]等4因素，与7项胁变指标的相关系数均达极显著水平，从总相关程度来看，缓冲量最高，其次是[CO_2^{2-}]、盐浓度和[Na^+]。在7项胁变指标中只有*RGR*和分蘖率与盐浓度的相关系数最高，其余各项的最大相关因素皆是缓冲量。可见碱性盐对胁变具有决定性作用。在诸胁迫因素中，[SO_4^{2-}]与各项胁变的相关性最低，除与*RGR*的相关系数达显著水平外，其余相关系数都很低，因而其致胁变作用可以忽略。在[Cl^-]和7项胁变指标间的相关系数中，有1项达极显著水平，3项达显著水平；在pH与7项胁变指标间的相关系数中有3项达极显著水平，3项达显著水平，所以[Cl^-]和pH的作用不可忽略。从各因素之间的相关性来看（表4中未列出），本实验中盐度和[Na^+]完全正相关（相关系数为1），而且二者与各项胁变指标间的相关系数也一致，所以[Na^+]完全可由盐浓度代表。缓冲量取决于[CO_3^{2-}]和[HCO_3^-]，若以2[CO_3^{2-}]＋[HCO_3^-]表示碱性盐总强度，缓冲量与其相关系数为0.9956，可见[CO_3^{2-}]和[HCO_3^-]完全可由缓冲量代表。

2.4 胁迫因素与数项胁变指标间的多元回归分析

根据上述相关性的分析结果可知，用缓冲量、盐度、pH和[Cl^-] 等4种因素即可代表所有的胁迫作用

因素。因为缓冲量代表了[HCO_3^-]和[CO_3^{2-}]的作用；盐度既代表了[Na^+]的作用也代表了渗透等其他作用；[SO_4^{2-}]的作用可以忽略。

以上述 4 因素为自变量，并设 x_1＝缓冲量、x_2＝盐度、x_3＝pH、x_4＝[Cl^-]，$X=b_1x_1+b_2x_2+b_3x_3+b_4x_4$。以各个胁变指标为因变量 Y，即 Y 分别等于 RGR 等。按公式 $Y=a+X$ 对各胁变指标进行多元线性回归。算出各自变量的标准回归系数 b' 并以此 比较各因素的重要性。计算出全相关系数平方即 R^2 以评价各回归方程的回归效果。

表 2　各处理的胁迫因素数据

Table 2　Data of stress factors for various treatments

处理 Treatment	胁迫因素 Stress factors							
	pH	盐度 Salinity (mmol/L)	缓冲量 Buffer capacity (H^+mmol)	[Na^+] (mmol/L)	[Cl^-] (mmol/L)	[SO_4^{2-}] (mmol/L)	[HCO_3^-] (mmol/L)	[CO_3^{2-}] (mmol/L)
A1	7.14	50	0.01	75.0	25.00	25.00	0.00	0.00
A2	7.18	125	0.02	187.5	62.50	62.50	0.00	0.00
A3	7.19	200	0.03	300.0	100.00	100.00	0.00	0.00
A4	7.26	275	0.04	415.5	137.50	137.50	0.00	0.00
A5	7.31	350	0.05	525.0	175.00	175.00	0.00	0.00
B1	7.95	50	8.00	75.0	12.50	25.00	12.50	0.00
B2	8.13	125	26.50	187.5	31.25	62.50	31.25	0.00
B3	8.18	200	33.50	300.0	50.00	100.00	50.00	0.00
B4	8.23	275	42.50	412.5	68.75	137.50	68.75	0.00
B5	8.44	350	62.00	525.0	87.50	175.00	87.50	0.00
C1	8.55	50	19.80	75.0	2.50	22.50	22.50	2.50
C2	8.77	125	48.40	187.5	6.25	56.25	56.25	6.25
C3	8.79	200	76.00	300.0	10.00	90.00	90.00	10.00
C4	8.85	275	107.00	412.5	13.75	123.80	123.80	13.75
C5	8.94	350	144.00	525.0	17.50	157.50	157.50	17.50
D1	9.58	50	28.90	75.0	12.50	12.50	12.50	12.50
D2	9.69	125	72.30	187.5	31.25	31.25	31.25	31.25
D3	9.77	200	113.00	300.0	50.00	50.00	50.00	50.00
D4	9.92	275	166.00	412.5	68.75	68.75	68.75	68.75
D5	9.96	350	217.00	525.0	87.50	87.50	87.50	87.50
E1	10.30	50	43.90	75.0	22.50	2.50	2.50	22.50
E2	10.48	125	103.00	187.5	56.25	6.25	6.25	56.25
E3	10.49	200	167.00	300.0	90.00	10.00	10.00	90.00
E4	10.50	275	216.00	412.5	123.80	13.75	13.75	123.80
F1	10.61	50	50.20	75.0	2.50	2.50	22.50	22.50
F2	10.63	125	134.00	187.5	6.25	6.25	56.25	56.25
F3	10.71	200	227.00	300.0	10.00	10.00	90.00	90.00

2.4.1　RGR **回归分析**　从图 2 可见 RGR 与 4 因素间回归方程的全相关系数为 0.9581，这表明 4 因素与 RGR 间具有高度线性相关性。此分析结果以及其他胁变指标的分析结果均可证明：用缓冲量、盐度、pH 及[Cl^-]即可有效地代表复合盐胁迫作用的诸因素。比较各因素的标准回归系数可知，对 RGR 这一胁变指标来说，各因素的重要性依次为盐度、缓冲量、pH 和[Cl^-]，其中盐度和缓冲量为决定性的主导因素，而 pH 和[Cl^-]的作用较小。若去掉 pH 或[Cl^-]进行三元回归，其回归方程的全相关系数分别为 0.9549 和 0.9523，这进一步证明二者对 RGR 的影响较小。

2.4.2　**分蘖率回归分析**　如图 2 一样，图 3 更明显地表明 4 因素与分蘖率间高度线性相关。各因素对分蘖率的重要性依次为盐度、pH、缓冲量和[Cl^-]，其中盐度是决定性的主导因素其次是 pH 和缓冲量，[Cl^-]的作用很小，去掉[Cl^-]后再进行三元回归分析得 $R^2=0.9647$ 与四元回归的效果基本一致，可见[Cl^-]对分

蘖率的作用小至可以忽略。

表 3 各处理的胁变值数据

Table 3 Data of strain values for various treatments

处理 Treatment	胁变值 Strain values						
	RGR (%)	分蘖率(条/株) Tillering rate (number/plant)	根茎数(条/株) Rhizome number (number/plant)	Na^+含量 Na^+ content (mmol/g dw)	K^+含量 K^+ content (mmol/g dw)	脯氨酸含量 Proline content (μmol/g dw)	电解质外渗率 Electrolyte leakage rate (%)
A1	105.0	1.28	0.88	0.23	0.75	4.34	7.26
A2	91.9	1.10	0.71	0.31	0.74	4.74	7.49
A3	74.1	0.86	0.64	0.38	0.71	11.56	9.19
A4	39.0	0.71	0.61	0.50	0.66	16.28	11.13
A5	11.1	0.53	0.49	0.75	0.58	46.97	23.30
B1	100.7	1.23	0.85	0.24	0.74	5.10	7.24
B2	88.3	0.96	0.69	0.34	0.71	5.92	9.00
B3	52.0	0.90	0.63	0.39	0.69	14.45	11.38
B4	34.8	0.62	0.52	0.51	0.63	18.52	17.05
B5	4.1	0.45	0.44	0.79	0.51	50.58	27.15
C1	99.3	1.19	0.78	0.25	0.73	5.42	8.59
C2	84.5	0.89	0.57	0.36	0.70	8.81	10.55
C3	44.9	0.68	0.53	0.41	0.60	17.52	13.05
C4	22.7	0.48	0.49	0.56	0.56	26.19	27.40
C5	−2.9	0.35	0.39	0.85	0.49	56.45	37.55
D1	93.0	0.99	0.70	0.28	0.71	6.10	10.00
D2	73.6	0.91	0.59	0.44	0.65	9.94	12.50
D3	26.7	0.66	0.39	0.74	0.55	26.19	19.40
D4	9.2	0.27	0.20	1.10	0.51	65.93	36.30
D5	−10.7	0.06	0.11	1.14	0.46	94.84	51.00
E1	92.4	0.91	0.67	0.29	0.68	6.77	10.90
E2	58.1	0.84	0.49	0.61	0.58	24.39	22.90
E3	−1.1	0.46	0.30	0.84	0.49	52.84	38.70
E4	−10.8	0.16	0.20	1.13	0.46	90.32	49.40
F1	86.2	0.82	0.62	0.30	0.66	7.77	11.70
F2	57.3	0.70	0.43	0.69	0.55	35.22	25.30
F3	−5.7	0.33	0.14	1.16	0.44	71.80	51.30

表 4 各胁迫作用因素与各胁变指标间的相关系数

Table 4 Correlation coefficients between stress factors and strain indices

胁迫因素 Stress factor	胁变指标 Strain index						
	RGR	分蘖率 Tillering rate	根茎数 Rhizome number	Na^+含量 Na^+ content	K^+含量 K^+ content	脯氨酸含量 Proline content	电解质外渗率 Electrolyte leakage rate
pH	−0.3185	−0.4470	−0.5978	0.4857	−0.5907	0.4478	0.5614
盐度 Salinity	−0.8778	−0.8233	−0.6748	0.7149	−0.6971	0.7088	0.6303
缓冲量 Buffer capacity	−0.7474	−0.8086	−0.9092	0.8734	−0.8937	0.8550	0.9270
[Na^+]	−0.8774	−0.8228	−0.6741	0.7143	−0.6963	0.7080	0.6294
[Cl^-]	−0.4921	−0.4143	−0.3106	0.4031	−0.2948	0.4155	0.2693
[SO_4^{2-}]	−0.4234	−0.3323	−0.1013	0.1515	−0.1537	0.1541	0.0496
[HCO_3^-]	−0.5409	−0.5586	−0.4892	0.4380	−0.5554	0.4170	0.4796
[CO_3^{2-}]	−0.6017	−0.6654	−0.8124	0.7918	−0.7627	0.7788	0.8290

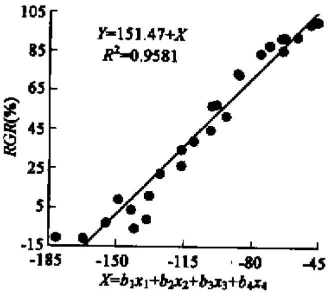

图 2　*RGR* 四元线性回归图

Fig. 2　Multiple regression between *RGR* and four factors

X_1＝缓冲量 Buffer capacity；X_2＝盐度 Salinity；X_3＝pH；X_4＝[Cl⁻]；b_1＝－0.21177；b_2＝－0.25431；b_3＝－3.79431；b_4＝－0.09105b'_1＝－0.38422；b'_2＝－0.62288；b'_3＝－0.11694；b'_4＝－0.10519

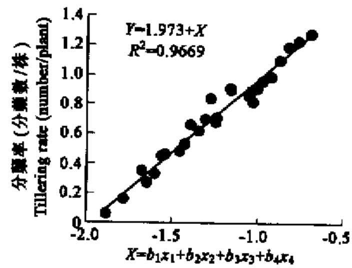

图 3　分蘖率四元线性回归图

Fig. 3　Multiple regression between tillering rate and the four factors

X_1＝缓冲量 Buffer capacity；X_2＝盐度 Salinity；X_3＝pH；X_4＝[Cl⁻]；b_1＝－0.0013；b_2＝－0.00215；b_3＝－0.08117；b_4＝－0.00045b'_1＝－0.290268；b'_2＝－0.689685；b'_3＝－0.307883；b'_4＝－0.063981

2.4.3　根茎回归分析　图 4 同样显示出根茎数与 4 因素间高度线性相关。各因素的重要性依次为：缓冲量、盐度、pH 和[Cl⁻]，其中以缓冲量为决定性主导因素其次是盐度。[Cl⁻]的作用最小，去掉它进行三元回归得 R^2＝0.9472，可见[Cl⁻]对根茎的影响很小。

2.4.4　Na^+ 含量回归分析　首先对 Na^+ 含量进行四元回归分析，得回归结果为（图 5）：Y＝0.192＋0.003393×缓冲量＋0.000632×盐度－0.00896×pH＋0.001663×[Cl⁻]，R^2＝0.9469，4 因素的标准回归系数分别为 b'_1＝0.810255、b'_2＝0.216826、b'_3＝－0.036348、b'_4＝0.252881。据此可知 pH 对 Na^+ 含量的作用很小而且系数为负，将其去掉后进行三元回归，结果基本不变。在四因素中缓冲量是决定 Na^+ 含量的主导因素，其次是盐度和[Cl⁻]，pH 可以忽略。

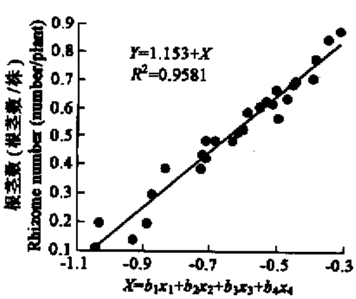

图 4　根茎四元线性回归图

Fig. 4　Multiple regression between rhizome number and the four factors

X_1＝缓冲量 Buffer capacity；X_2＝盐度 Salinity；X_3＝pH；X_4＝[Cl⁻]；b_1＝－0.00167；b_2＝－0.00071；b_3＝－0.03742；b_4＝－0.00065 b'_1＝－0.589607；b'_2＝－0.360132；b'_3＝－0.224432；b'_4＝－0.146132

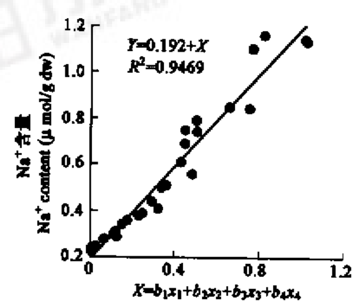

图 5　钠含量四元线性回归图

Fig. 5　Multiple regression between Na^+ content and the four factors

X_1＝缓冲量 Buffer capacity；X_2＝盐度 Salinity；X_3＝pH；X_4＝[Cl⁻]；b_1＝0.003393；b_2＝0.000632；b_3＝－0.00896；b_4＝0.001663 b'_1＝0.810255；b'_2＝0.216826；b'_3＝－0.036348；b'_4＝0.252881

2.4.5 K$^+$含量的回归分析 K$^+$含量四元回归结果为(图 6):$Y=0.986-0.000604\times$缓冲量$-0.000502\times$盐度$-0.025442\times pH-0.000067\times[Cl^-]$;$R^2=0.9518$;$b'_1=-0.436098$、$b'_2=-0.522108$、$b'_3=-0.314113$、$b'_4=-0.031009$。据此可知$[Cl^-]$是可忽略的因素,将其去掉后进行三元回归分析,效果与四元回归基本相同。3 因素中,盐度和缓冲量的重要性相近,都起主导作用,其次是 pH。

2.4.6 脯氨酸回归分析 脯氨酸含量四元回归的结果如下(图 7):$Y=14.833+0.339094\times$缓冲量$+0.039204\times$盐度$-3.06696\times pH+0.165466\times[Cl^-]$;$R^2=0.9246$;$b'_1=0.897197$、$b'_2=0.149024$、$b'_3=-0.138750$、$b'_4=0.278781$。因 pH 是最次要因素而且系数为负,将其去掉进行三元回归分析,效果与四元回归基本相同;脯氨酸含量与 4 因素间具有高度拟合的线性关系。4 因素中缓冲量是影响脯氨酸含量的决定性主导因素,其次是$[Cl^-]$和盐度,而 pH 可以忽略。

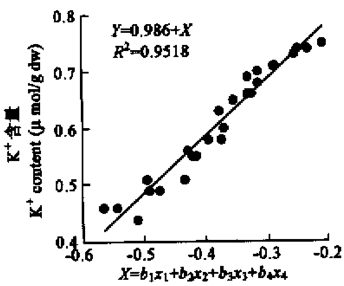

图 6 钾含量四元线性回归图

Fig. 6 Multiple regression between K$^+$ content and the four factors

$X_1=$缓冲量 Buffer capacity;$X_2=$盐度 Salinity;$X_3=$pH;$X_4=[Cl^-]$;$b_1=-0.000604$;$b_2=-0.000502$;$b_3=-0.025442$;$b_4=-0.0000674$ $b'_1=-0.436098$;$b'_2=-0.522108$;$b'_3=-0.314113$;$b'_4=-0.031009$

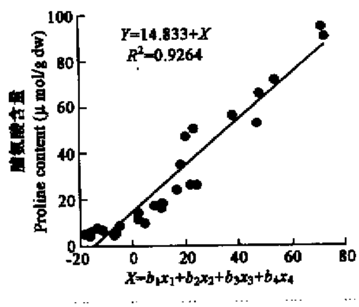

图 7 脯氨酸四元线性回归图

Fig. 7 Multiple regression between proline content and the four factors

$X1=$缓冲量 Buffer capacity;$X_2=$盐度 Salinity;$X_3=$pH;$X_4=[Cl^-]$;$b_1=0.339094$;$b_2=0.039204$;$b_3=-3.08696$;$b_4=0.165466 b'_1=0.897197$;$b'_2=0.149024$;$b'_3=-0.13875$;$b'_4=0.278781$

2.4.7 电解质外渗率的回归分析 叶片电解质外渗率四元回归的结果如下:$Y=11.061+0.186847\times$缓冲量$+0.018157\times$盐度$-1.15631\times pH+0.047751\times[Cl^-]$;$R^2=0.9468$;$b'_1=0.946993$、$b'_2=0.132209$、$b'_3=0.099557$、$b'_4=0.154110$。因 pH 是最次要因素 而且系数为负,所以去掉后进行三元回归分析,其回归结果与四元回归基本相同(图 8)。很显然,缓冲量是决定电解质外渗率的主导因素,其次是盐度和$[Cl^-]$。

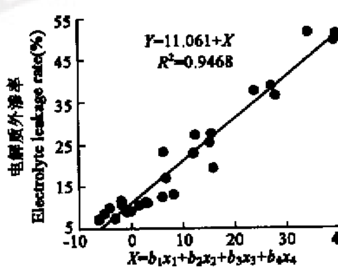

图 8 电解质外渗率四元线性回归图

Fig. 8 Multiple regression between electrolyte leakage rate and the four factors

$X_1=$缓冲量 Buffer capacity;$X_2=$盐度 Salinity;$X_3=$pH;$X_4=[Cl^-]$;$b_1=0.186847$;$b_2=0.018157$;$b_3=-1.15631$;$b_4=0.047751 b'_1=0.946993$;$b'_2=0.132209$;$b'_3=-0.099557$;$b'_4=0.154110$

3 讨论

3.1 盐碱混合胁迫条件的人工模拟

天然盐碱条件异常复杂影响因素众多且无法控制,因而极大地限制了对其研究,以至迄今为止对盐碱混合胁迫仍没有明显进展。要想通过科学实验揭示复杂盐碱混合胁迫这一难题的话,首先得创造出切实可行的研究方法。本实验依据天然盐碱地盐分组成特点[19],将 NaCl、$NaHCO_3$、Na_2SO_4 和 Na_2CO_3 按不同比例混合,模拟出数种盐度和 pH 各不相同的盐碱生态条件。结果表明:30 种处理均匀覆盖了总盐度 50~

350 mmol/L，pH 7.14～10.81 范围内的各种盐碱条件。本模拟方法可使复杂的盐碱条件再现并完全置于人工控制之下,这使对盐碱混合胁迫的研究有了可能,该方法实际应用的结果也充分证明了这一点。

3.2　盐碱混合胁迫的作用因素

对于天然盐碱混合生态条件来说,造成其复杂性的主要原因之一就是其所含盐分的组成、比例及数量各不相同。不同盐的性质不同,对植物的作用也不同,不同盐尤其是中性盐和碱性盐混合在一起其作用因素要比单一的中性盐[1~3]或碱性盐[4~7]复杂得多。一般认为,中性盐 NaCl 的胁迫作用因素主要是,以 Na^+ 为主的离子效应和高浓度盐造成水势下降的渗透效应[1],而碱性盐 Na_2CO_3 的作用因素则要在前者的基础上加上高 pH[4~7]。各种中、碱性盐混合在一起后,由于离子之间相互作用等原因,其作用因素绝非是二者因素加和那么简单。鉴于此,必须对盐碱混合胁迫的作用因素加以实验分析。

从对模拟出的 30 种盐碱混合生态条件所进行的胁迫因素分析中不难看出,缓冲量概念的引入对问题的简化起到了关键作用。缓冲量既可代表两种碱性盐的浓度与比例也可代表两种碳酸根的浓度与比例。所以最后的分析结果表明:用缓冲量、盐度、pH 和[Cl^-]等 4 因素即可较真实全面地代表复合盐胁迫的所有作用因素。

3.3　不同胁迫因素与胁变指标间的关系

对不同胁变指标来说,由于植物体的产生机制有所不同,所以不同作用因素对其决定程度也有所不同,但从 7 项胁变指标回归分析的结果明显可以看出:缓冲量和盐度对所有胁变指标来说都是不可缺少的主导因素。虽然在 *RGR*、分蘖率和 K^+ 含量等 3 项指标上盐度的作用大于或近于缓冲量，但在另外 4 项胁变指标上缓冲量的作用明显大于盐度,可见,在盐碱混合胁迫下缓冲量是个重要的胁强指标。在所分析的 7 项胁变指标中,pH 和[Cl^-]的作用都处于较次要的地位,有时小至可以忽略。从回归分析的结果还可以发现,不同胁迫因素对不同胁变指标的影响程度有所不同。这一点可能与植物对胁迫响应的生理机制及胁变形成的生理过程有关,值得深入研究。

本文结果是对文献中所提出的,用盐度代表盐胁强用缓冲量代表碱胁强这一观点的有力支持[10]。对于天然盐分复杂的盐碱地来说,只有用盐度加缓冲量作为表示其盐碱强度的指标,才具有科学性。

3.4　盐碱混合胁迫的理想胁强指标

研究胁迫时很重要的一点是确定适宜胁强指标。对盐胁迫,一般可用盐浓度、[Na^+]或电导率代表胁强,对于碱胁迫可用缓冲量或 pH 代表胁强,但在盐碱混合胁迫下,这些指标都难以较全面地反映胁迫强度。根据以上分析,用盐度加缓冲量作为盐碱混合胁迫的胁强指标较为合理。图 9 展示了以盐度加缓冲量为胁强,羊草 *RGR* 随盐碱混合胁迫强度的变化。图 9 中的曲线是线性回归趋势线($R^2=0.9485$)。从图 9 可以看出,羊草 *RGR* 随盐碱混合胁迫强度的增大而下降的规律。与单独以盐度、pH 或缓冲量为胁强相比,以盐度加缓冲量代表盐碱混合胁迫的强度更为合理。

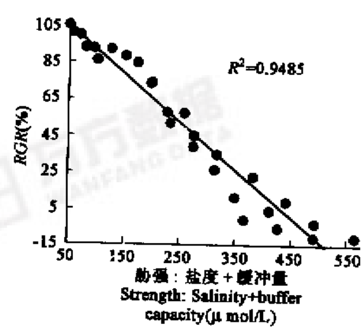

图 9　盐碱混合胁迫(以盐度加缓冲量为胁强)对羊草 RGR 的影响

Fig. 9　Effects of the strength (salinity + buffer capacity) of salt and alkali mixed stress on the *RGR of Aneurolepidium chinense*

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