

**Figure 9 Salt stress adaptation in regenerated plants in F2 and F3 generation.** Salt survival assay of the F2 and F3 progenies of control and salt-treated regenerated plants from leaf and root. The survival rates were analysed after 14 days on MS media with 150 mM NaCl by scoring plants with green cotyledons. Each line contains 50 seeds per plate with 6 technical replicates and five lines per plant type were used in this experiment. The survival rates (%) were normalised by dividing with the survival rates in MS media without salt to eliminate the deviation from germination rates. 75LOF2/F3, leaf-origin progenies regenerated from salt-treated plants in F2/F3 generation, 75ROF2/F3, root-origin progenies regenerated from salt-treated plants in F2/F3 generation, CLOF2/F3, leaf-origin progenies regenerate from control plants in F2/F3 generation, CROF2/F3, root-origin progenies from control plants in F2/F3 generation.

Commend in R PROGRAMM

library(ggplot2)

library(ggpubr)

library(tidyverse)

X15\_10\_18\_result\_salt\_tolerance

raw\_data\_salt\_survivor <- X15\_10\_18\_result\_salt\_tolerance

raw\_data\_salt\_survivor

raw\_data\_salt\_survivor$Media

####### Manipulate data frame

raw\_data\_salt\_survivor

raw\_data\_salt\_survivor <- rename(raw\_data\_salt\_survivor, replicate = '# Replicate')

raw\_data\_salt\_survivor <- rename(raw\_data\_salt\_survivor, percentage = '% Survivors')

raw\_data\_salt\_survivor <- rename(raw\_data\_salt\_survivor, line = Line, generation = Generation, media = Media)

raw\_data\_salt\_survivor

raw\_data\_salt\_survivor\_selected <- select(raw\_data\_salt\_survivor, line, replicate, generation, media, percentage)

raw\_data\_salt\_survivor\_selected

###### Seperating by media

normal\_MS <- filter(raw\_data\_salt\_survivor\_selected, media == "N")

normal\_MS

salt\_MS <- filter(raw\_data\_salt\_survivor\_selected, media == "S")

salt\_MS

normal\_MS$line == salt\_MS$line # check the accuracy of the line

# All TRUE

normalised\_survivor <- mutate(salt\_MS, normalised = (salt\_MS$percentage\*100)/normal\_MS$percentage)

normalised\_survivor

survivor\_graph <- ggplot(data = normalised\_survivor) +

geom\_boxplot(aes(x = line, y = normalised, fill = sub("\_\\d", "", line))) +

geom\_hline(yintercept = 50, color = "red") +

facet\_grid(generation~.) +

theme\_classic()

survivor\_graph

pdf("survival\_figure\_not\_collapsed.pdf", height = 5,width = 7 )

survivor\_graph

dev.off()

##### Making the survival\_percentage in normal media

normal\_MS

normal\_MS\_graph <- ggplot(data = normal\_MS) +

geom\_boxplot(aes(x = line, y = percentage, fill = sub("\_\\d", "", line))) +

geom\_hline(yintercept = 80, color = "red") +

facet\_grid(generation~.) +

theme\_classic()

normal\_MS\_graph

pdf("normal\_MS\_survivors.pdf", height = 5,width = 7 )

normal\_MS\_graph

dev.off()

# TRY geom\_boxplot

ggplot(data = normal\_MS) +

geom\_boxplot(aes(x = line, y = percentage)) + # without 'fill = ' it still combine 'each line' together

geom\_hline(yintercept = 80, color = "red") +

facet\_grid(generation~.) +

theme\_classic()

# aes(x=line) combines CROF2\_1 from all replicates together

salt\_MS\_graph <- ggplot(data = salt\_MS) +

geom\_boxplot(aes(x = line, y = percentage, fill = sub("\_\\d", "", line))) + # without 'fill = ' it still combine 'each line' together

geom\_hline(yintercept = 50, color = "red") +

facet\_grid(generation~.) +

theme\_classic()

pdf("salt\_MS\_survivors.pdf", height = 5,width = 7 )

salt\_MS\_graph

dev.off()

DATA

line generation replicate survivors total percentage

Col\_0 F2 1 48 49 97.96

Col\_0 F2 2 50 51 98.04

Col\_0 F2 3 49 50 98.00

Col\_0\_1 F2 1 47 50 94.00

Col\_0\_1 F2 2 47 50 94.00

Col\_0\_1 F2 3 49 50 98.00

CROF2\_1 F2 1 44 50 88.00

CROF2\_1 F2 2 44 50 88.00

CROF2\_1 F2 3 39 50 78.00

CROF2\_1 F2 4 NA NA NA

CROF2\_2 F2 1 45 50 90.00

CROF2\_2 F2 2 39 50 78.00

CROF2\_2 F2 3 43 50 86.00

CROF2\_2 F2 4 46 50 92.00

CROF2\_3 F2 1 40 50 80.00

CROF2\_3 F2 2 44 50 88.00

CROF2\_3 F2 3 43 50 86.00

CROF2\_3 F2 4 42 50 84.00

CROF2\_4 F2 1 41 50 82.00

CROF2\_4 F2 2 43 51 84.31

CROF2\_4 F2 3 47 50 94.00

CROF2\_4 F2 4 47 50 94.00

CROF2\_5 F2 1 43 50 86.00

CROF2\_5 F2 2 46 50 92.00

CROF2\_5 F2 3 43 50 86.00

CROF2\_5 F2 4 43 50 86.00

CLOF2\_1 F2 1 48 50 96.00

CLOF2\_1 F2 2 48 50 96.00

CLOF2\_1 F2 3 44 50 88.00

CLOF2\_1 F2 4 NA NA NA

CLOF2\_2 F2 1 49 50 98.00

CLOF2\_2 F2 2 40 50 80.00

CLOF2\_2 F2 3 47 50 94.00

CLOF2\_2 F2 4 45 50 90.00

CLOF2\_3 F2 1 35 40 87.50

CLOF2\_3 F2 2 46 50 92.00

CLOF2\_3 F2 3 44 50 88.00

CLOF2\_3 F2 4 43 50 86.00

CLOF2\_4 F2 1 48 50 96.00

CLOF2\_4 F2 2 48 50 96.00

CLOF2\_4 F2 3 45 50 90.00

CLOF2\_4 F2 4 46 50 92.00

CLOF2\_5 F2 1 44 50 88.00

CLOF2\_5 F2 2 44 50 88.00

CLOF2\_5 F2 3 49 50 98.00

CLOF2\_5 F2 4 48 50 96.00

75ROF2\_6 F2 1 47 50 94.00

75ROF2\_6 F2 2 47 50 94.00

75ROF2\_6 F2 3 45 50 90.00

75ROF2\_6 F2 4 49 50 98.00

75ROF2\_2 F2 1 43 50 86.00

75ROF2\_2 F2 2 49 50 98.00

75ROF2\_2 F2 3 48 50 96.00

75ROF2\_2 F2 4 48 50 96.00

75ROF2\_3 F2 1 43 50 86.00

75ROF2\_3 F2 2 51 51 100.00

75ROF2\_3 F2 3 42 50 84.00

75ROF2\_3 F2 4 44 50 88.00

75ROF2\_4 F2 1 41 50 82.00

75ROF2\_4 F2 2 39 50 78.00

75ROF2\_4 F2 3 43 50 86.00

75ROF2\_4 F2 4 45 50 90.00

75ROF2\_5 F2 1 39 50 78.00

75ROF2\_5 F2 2 42 50 84.00

75ROF2\_5 F2 3 44 50 88.00

75ROF2\_5 F2 4 46 51 90.20

75LOF2\_1 F2 1 47 50 94.00

75LOF2\_1 F2 2 48 50 96.00

75LOF2\_1 F2 3 NA NA NA

75LOF2\_1 F2 4 NA NA NA

75LOF2\_2 F2 1 50 50 100.00

75LOF2\_2 F2 2 49 50 98.00

75LOF2\_2 F2 3 49 50 98.00

75LOF2\_2 F2 4 51 51 100.00

75LOF2\_3 F2 1 47 50 94.00

75LOF2\_3 F2 2 50 50 100.00

75LOF2\_3 F2 3 48 50 96.00

75LOF2\_3 F2 4 47 50 94.00

75LOF2\_4 F2 1 49 50 98.00

75LOF2\_4 F2 2 50 50 100.00

75LOF2\_4 F2 3 50 50 100.00

75LOF2\_4 F2 4 50 50 100.00

75LOF2\_5 F2 1 49 50 98.00

75LOF2\_5 F2 2 48 50 96.00

75LOF2\_5 F2 3 50 50 100.00

75LOF2\_5 F2 4 48 50 96.00

CROF3\_1 F3 1 47 49 95.92

CROF3\_1 F3 2 48 50 96.00

CROF3\_1 F3 3 49 50 98.00

CROF3\_2 F3 1 48 50 96.00

CROF3\_2 F3 2 50 50 100.00

CROF3\_2 F3 3 49 50 98.00

CROF3\_3 F3 1 49 50 98.00

CROF3\_3 F3 2 50 50 100.00

CROF3\_3 F3 3 49 50 98.00

CROF3\_4 F3 1 50 50 100.00

CROF3\_4 F3 2 50 50 100.00

CROF3\_4 F3 3 49 50 98.00

CROF3\_5 F3 1 49 50 98.00

CROF3\_5 F3 2 46 50 92.00

CROF3\_5 F3 3 48 50 96.00

CLOF3\_1 F3 1 50 50 100.00

CLOF3\_1 F3 2 50 50 100.00

CLOF3\_1 F3 3 48 50 96.00

CLOF3\_2 F3 1 49 50 98.00

CLOF3\_2 F3 2 47 50 94.00

CLOF3\_2 F3 3 48 50 96.00

CLOF3\_3 F3 1 49 50 98.00

CLOF3\_3 F3 2 50 50 100.00

CLOF3\_3 F3 3 50 50 100.00

CLOF3\_4 F3 1 51 51 100.00

CLOF3\_4 F3 2 49 50 98.00

CLOF3\_4 F3 3 47 50 94.00

CLOF3\_5 F3 1 50 50 100.00

CLOF3\_5 F3 2 50 50 100.00

CLOF3\_5 F3 3 47 50 94.00

75ROF3\_6 F3 1 48 50 96.00

75ROF3\_6 F3 2 49 53 92.45

75ROF3\_6 F3 3 48 50 96.00

75ROF3\_2 F3 1 48 50 96.00

75ROF3\_2 F3 2 48 50 96.00

75ROF3\_2 F3 3 50 50 100.00

75ROF3\_3 F3 1 45 50 90.00

75ROF3\_3 F3 2 48 50 96.00

75ROF3\_3 F3 3 48 50 96.00

75ROF3\_4 F3 1 46 50 92.00

75ROF3\_4 F3 2 48 50 96.00

75ROF3\_4 F3 3 49 50 98.00

75ROF3\_5 F3 1 50 51 98.04

75ROF3\_5 F3 2 49 50 98.00

75ROF3\_5 F3 3 50 52 96.15

75LOF3\_1 F3 1 48 50 96.00

75LOF3\_1 F3 2 48 50 96.00

75LOF3\_1 F3 3 48 50 96.00

75LOF3\_2 F3 1 49 50 98.00

75LOF3\_2 F3 2 49 50 98.00

75LOF3\_2 F3 3 49 50 98.00

75LOF3\_3 F3 1 49 50 98.00

75LOF3\_3 F3 2 48 50 96.00

75LOF3\_3 F3 3 50 50 100.00

75LOF3\_4 F3 1 48 50 96.00

75LOF3\_4 F3 2 50 50 100.00

75LOF3\_4 F3 3 49 50 98.00

75LOF3\_5 F3 1 50 50 100.00

75LOF3\_5 F3 2 49 50 98.00

75LOF3\_5